

Biology

Teaching Resource

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**Molecules, Cells &
Systems**

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Molecules, Cells and Systems

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Molecules, Cells and Systems

1.1

The cell as the basic unit of structure in prokaryotic and eukaryotic organisms

Contents

Prokaryotic cells

- ▼ The main features of prokaryotic cells

Eukaryotic cells

- ▼ The structure of plant and animal cells as seen through an optical microscope
- ▼ The preparation of temporary mounts, the use of simple staining techniques (see practical work) and the estimation of size (also see practical work)

Introduction

All living organisms are composed of cells. It is calculated that an average human adult is made up of around 10^{14} cells, each coordinated with the others to form functional tissues, organs and systems. At the other extreme, some organisms are composed of a single cell; Amoeba, bacteria and yeast are examples of single celled organisms.

The word 'cell' to describe living units was first coined by the English Scientist Robert Hooke who, in the 1660s, used a simple microscope to observe the contents of a slice of cork from the bark of a tree. He noted that the structure resembled a multitude of tiny compartments like the cells inhabited by monks in a monastery - hence the term 'cell'.

Cells are grouped into two major types, **prokaryotic** and **eukaryotic**, the most important distinction between them being the presence or absence of a nucleus. The word '*karyon*' in Greek means 'nucleus', '*eu*' means true, and '*pro*' means 'before'. Eukaryotic cells are so named because they have a true nucleus and prokaryotic cells are built on an earlier design without a true nucleus.

PROKARYOTIC CELLS

The earliest life forms on the planet were prokaryotes similar in structure to present day bacteria. Prokaryotes are all single celled organisms and they are classified in a separate kingdom called **Kingdom Prokaryotae** (formerly, Monera). Although they are all constructed according to the same basic pattern, they are a very diverse group with individual types capable of inhabiting parts of the earth as distinct from each other as hot sulphurous springs and permafrost. Some prokaryotes are photosynthetic and may occur in such numbers as to form a blue green scum on the surface of stagnant water. Others (decomposers) live off the dead remains of other organisms, assisting in the breakdown, decay and recycling of essential nutrients. A few prokaryotes have gained notoriety as disease agents (pathogens) due to their parasitic life style e.g. *Mycobacterium tuberculosis*, *Salmonella enteritidis*.

Prokaryotic cells are much smaller than eukaryotic cells, ranging from 1-10 μm in size (eukaryotic cells range from 10-100 μm). Like eukaryotic cells, prokaryotes are capable of carrying out the life processes of sensitivity (reacting to different stimuli e.g. temperature and interacting with other cells), movement (all living cells show streaming of the cytoplasm even if the cell itself does not move), nutrition, respiration (release and use energy from energy rich substances), excretion (elimination of toxic waste products), growth (increase in size and complexity), and reproduction.

Not all prokaryotes are able to move, but some possess **flagella**, which are like elongated cilia (but without the microtubules - see 10.2), capable of propelling the organism through water. The cell wall is not made of cellulose, but is formed from a mixture of polysaccharides and amino acids called **murein**. Many bacteria which cause disease (pathogenic bacteria) have a **slime capsule** as their outermost layer which helps in protection against the body defences of the infected organism.

Prokaryotic cells generally divide and reproduce themselves much more quickly than eukaryotic cells. When grown in ideal conditions, a typical bacterial cell can grow and divide every 20 minutes, so a single cell could theoretically give rise to 2^{36} (that is a 2 with 36 noughts) in just 12 hours!

The structure of a generalised bacterial cell is illustrated and described in section 10.2 which also lists the main differences between prokaryotic and eukaryotic cells in the form of a table.

◆ CHECKPOINT SUMMARY

- ◆ Prokaryotic cells (bacteria) lack a true nucleus and membrane bound organelles
- ◆ The DNA is located in a central strand, and as circular plasmids in the rest of the cytoplasm
- ◆ The cell wall is not cellulose as in plant cells.
- ◆ Respiration is located in mesosomes which are infoldings of the cell surface membrane, showing the principle of increased surface area of membranes with true mitochondria
- ◆ The origin of mitochondria and chloroplasts as mutualistic prokaryotes with eukaryote cells, explains their absence in prokaryotes
- ◆ Ribosomes present in both
- ◆ Prokaryotic cells may form colonies but never multicellular organisms/tissues.

EUKARYOTIC CELLS

The structure of plant and animal cells as seen through an optical microscope.

Hooke used the word cell to mean a small compartment. When a slice of tissue from a plant or animal is viewed through a microscope, it is easy to see how this idea originated. Plant cells are generally easier to see under the microscope, as the cell membrane of each cell is surrounded by a relatively thick **cellulose cell wall** which gives each cell a more visible boundary.

The plant cell wall is made of a fibrous cellulose framework mixed with other materials, notably **pectin**. Pectin is a glue like material (it is used to make jam set) and it is concentrated on the surface, sticking adjacent

cells together. This sticky region can be seen as a line on electron micrographs and is called the middle lamella. When stretched around an expanded (turgid) cell, the cell wall makes a considerable contribution to the support of non-woody structures, e.g. leaves. The cell wall is fully permeable to water and dissolved solutes, but influences the exchange of these across the partially permeable cell membrane, by exerting various physical and chemical forces. The wall is perforated by tiny holes, through which strands of cytoplasm (**plasmodesmata**) pass giving direct communication between neighbouring cells.

In 'woody' parts the cell wall is impregnated with **lignin**. Lignin is a hard, impermeable substance, and lignification typically results in the death of the cell. Such dead lignified tissues are specialised for water transport (e.g. xylem) and support (e.g. xylem and sclerenchyma fibres).

As cells in a multicellular organism become more specialised (differentiated) for particular activities e.g. transmission of impulses in nerve cells, they lose the ability to carry out some of the life processes, e.g. under normal circumstances most nerve cells lose the ability to divide. However, if some of these differentiated cells are removed from the body and kept isolated in a special culture solution they are capable of surviving on their own, and may even regain some of the lost abilities, such as the ability to divide.

Multicellular organisms may also produce single cells which can live independently, e.g. spermatozoa.

Cells which lose their nucleus cannot survive or divide, because the nucleus controls all the life processes. If an Amoeba is cut into two, the part without the nucleus dies, but the part with the nucleus lives, grows and reproduces.

Cheek lining (squamous) cell under light microscope

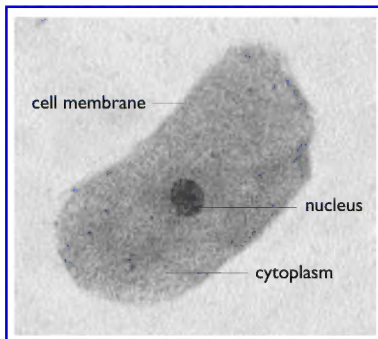
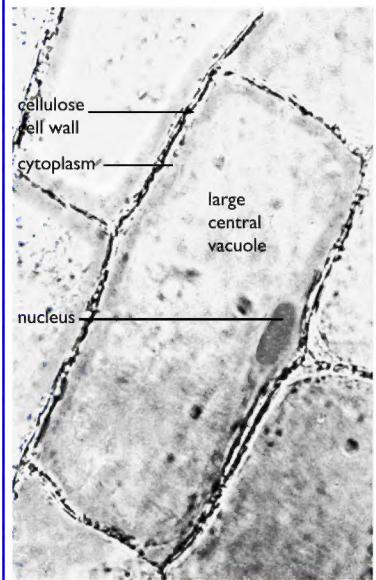


Table of differences between typical animal and plant cells seen under the light microscope.

Animal cells	Plant cells
Cell surface membrane	Cell surface membrane secretes cellulose cell wall
Small scattered vacuoles	Large central vacuole surrounded by membrane (tonoplast)
No chloroplasts	Chloroplasts with starch grains (in cells exposed to light)

Onion epidermis cells under light microscope



◆ CHECKPOINT SUMMARY

- ◆ Eukaryotes do have a true membrane bound nucleus containing chromosomes of DNA and histone proteins, and membrane bound organelles as described previously
- ◆ Eukaryotic cells typically form tissues in multicellular organisms
- ◆ Cell differentiation and specialisation prevent the easy definition of typical animal and plant cells
- ◆ 'Typical' cells would be those considered to show features common to the vast majority of cells in animals or plants
- ◆ A squamous (pavement) epithelium cell from the lining of the mouth could be considered as a 'typical' animal cell
- ◆ A mesophyll cell from a leaf could be considered as a 'typical' plant cell
- ◆ Under the best light microscope both the typical animal cell and the typical plant cell can be seen to possess a cell surface membrane, cytoplasm, nucleus with nucleolus, mitochondria, endoplasmic reticulum, and Golgi body
- ◆ In addition the typical plant cell can be seen to possess a cell wall around the cell surface membrane, a large central vacuole surrounded by the tonoplast membrane, and chloroplasts.

Estimating the size when observing cells through a microscope

When looking at objects under the light microscope the magnification of the object as you see it can be calculated by multiplying the magnifying power of the eyepiece lens and the objective lens (the lens at the bottom) to get the overall magnification, for example $\times 10$ and $\times 40$ respectively under high power giving a total magnification of $\times 400$. However, when you make a drawing of a specimen seen under the light microscope, the size of your drawing further magnifies the image. To calculate the linear magnification (e.g. the number of times the length of an object has been magnified) of drawings it is necessary to measure your drawing and calculate how many times it is greater than the actual size of the object as seen under the microscope.

To calculate the actual size of the object you need to use a stage micrometer and an eye piece graticule. The stage micrometer is a microscope slide with a very fine scale marked on it. If it was possible to place a specimen on the scale it could be measured directly, but this is not easily done. Therefore a glass or plastic disc with a printed scale, known as an eye piece graticule is mounted in the microscope eyepiece. It does not have actual measurements on it, just lines. At each magnification the number of divisions on the eye piece graticule equal to a certain real measurement on the stage micrometer is noted. For example 10 eye piece divisions will equal a larger real measurement at $\times 100$ than at $\times 400$. Once this calibration has been carried out the eye piece graticule can be used to measure the specimen.

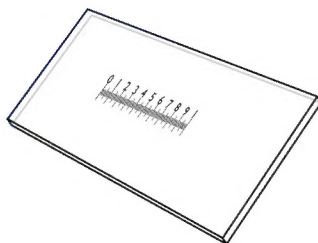
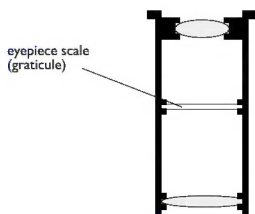
Cheek cells are about $50\text{ }\mu\text{m}$ in diameter, red blood cells $6\text{--}8\text{ }\mu\text{m}$, and some plant cells up to $500\text{ }\mu\text{m}$.

◆ CHECKPOINT SUMMARY

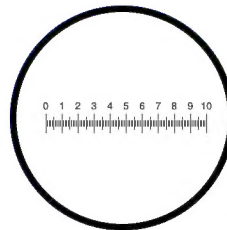
- ◆ If the specimen can be observed with the naked eye without the need for magnification the calculation of the linear magnification of any drawing of it is straightforward - being the number of times any linear dimension of the drawing is greater than that of the specimen
- ◆ Magnifications of images of slides of biological material are often given in terms relating to the magnification of the microscope image, e.g. $\times 100$ and $\times 400$
- ◆ However reproductions of the image as seen down a microscope introduce another layer of magnification which is often overlooked
- ◆ To calculate the linear magnification of drawings of microscope images of specimens it is necessary to know how many times the drawing is larger than the actual specimen
- ◆ The actual size of the specimen is measured with an eye piece graticule and stage micrometer.

Measuring with a microscope eyepiece graticule

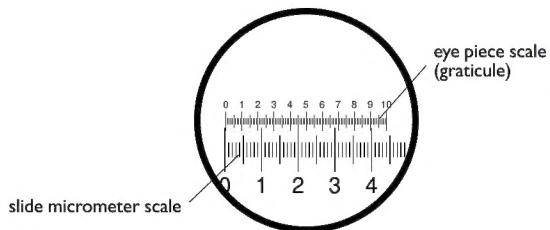
Eyepiece scale (graticule)



Scale in mm etched upon a microscope slide or a printed acetate sheet (slide micrometer)



Eyepiece scale as seen when held up to the light away from the microscope.



Accurate measurements can be achieved using the two scales in combination through the lenses of the microscope.

1.2

The electron microscope and the technique of cell fractionation in the study of ultrastructure

Contents

Electron microscopes

- ▼ The difference between magnification and resolution.
- ▼ The principles and limitations of transmission and scanning electron microscopes

Cell ultrastructure

- ▼ Interpretation of electron micrographs (see teaching guides)
- ▼ Identification of the principal features and organelles of a eukaryotic cell. Cell wall and plasma membranes, nucleus, chloroplasts, mitochondria, lysosomes, ribosomes, endoplasmic reticulum, Golgi apparatus, microvilli and vesicles.
- ▼ The functions of these structures
- ▼ The principal features of a bacterium. Cell wall, capsule and genetic material

Cell fractionation

- ▼ Principles of cell fractionation and ultracentrifugation as used to separate cell components

ELECTRON MICROSCOPES

The difference between magnification and resolution

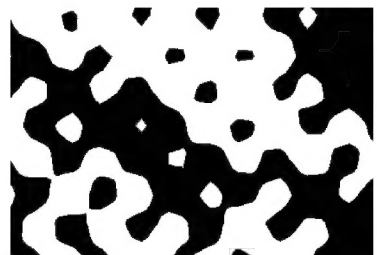
Magnification

For the visual study of cell structure it is necessary to enlarge (magnify) them by the use of microscopes. **Light microscopes** that you are most likely to come across in general Biology laboratories usually magnify up to 400 times (special techniques could take this up to 1000 times). However, to continue magnifying objects past a certain point reveals no further detail. In order to see more detail it is necessary to upgrade the resolving power (resolution).

Resolution

Resolution or resolving power can be defined as the ability of an optical system (e.g. the light microscope, the eye) to distinguish (resolve) detail in an image of an object.

The amount of detail that can be seen in an image is determined by the resolving power of the microscope system being used. In the case of looking at material under the light microscope the resolving power depends upon the light being able to distinguish this detail. To do



this light must be reflected back from structures. Any structures less than 0.2 micrometres (μm), that is 200 nanometres (nm) in diameter cannot be distinguished by light, nor can two structures closer than 0.2 μm be seen as separate.

(1 μm = one thousandth of a millimetre, 1 nm = one millionth of a millimetre)

To improve the resolution, it is necessary to have a device which uses a beam of electrons, instead of light, called the **electron microscope**. Electron beams have a much shorter wavelength than light and give a 100 times better resolution. The magnification of an electron microscope picture (electron micrograph) can also be increased significantly to reveal this extra detail. The most powerful electron microscope can magnify up to $\times 500\,000$.

The principles and limitations of transmission and scanning electron microscopes

There are two types of electron microscope. In the **transmission electron microscope (TEM)**, an extremely thin section of the specimen is held on a metal grid and an electron beam passes right through it. Inside the electron microscope tube, electrons are accelerated by high voltage and focussed into a fine beam by a strong electromagnet (condenser lens). The beam passes through the specimen and is focussed further by additional electromagnets, referred to as the objective, intermediate and projector lenses, to give an image on a fluorescent screen, similar to the screen of a television set.

Electrons are disrupted by particles in the specimen to produce lighter and darker areas on the screen which correspond to dense and less dense parts of the specimen. If a photographic film plate is substituted for the fluorescent screen, the resulting picture is called an **electron micrograph**. A vacuum is maintained inside the tube of the electron microscope so that the electron beam is not disrupted by molecules of the air e.g. oxygen and water. For this reason, it is impossible to view living specimens. This, along with the risk of the distortion of the delicate sub-microscopic structures (production of artefacts) by the preparation procedures are the principal limitations of electron microscope images. They show the structure of dead cells which have been subjected to mechanical and chemical trauma in the preparation process and are held in a vacuum.

Specimens are prepared by immersion in a series of increasingly pure solutions of alcohol which serve to dehydrate the cell components, then they are typically embedded in a block of resin which is cut into very thin sections by a machine resembling a high tech. bacon slicer called a microtome with a diamond or glass cutting surface. To improve the contrast of these pictures, it is common to 'stain' the specimen with the salts of heavy metals such as lead and uranium which increase the scattering of the electrons where the 'stains' are taken up.

In the **scanning electron microscope (SEM)**, the beam of electrons passes back and forth across the surface of the prepared specimen producing a three dimensional effect. This technique is used to show surface features.

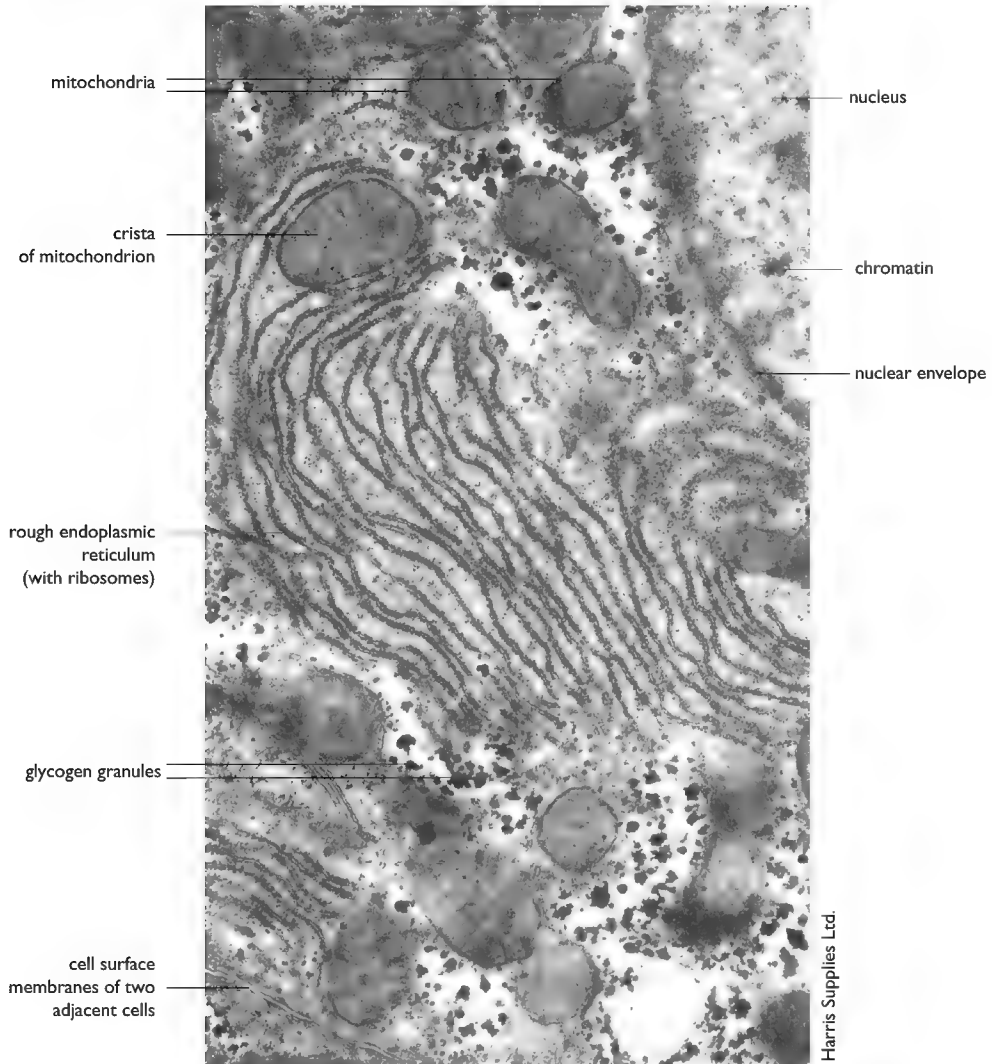
◆ CHECKPOINT SUMMARY

- ◆ Electron microscopes use a beam of electrons, instead of light. The electrons are focussed by magnets onto a fluorescent screen to produce an image which may be photographed to produce electron micrographs
- ◆ Electron beams have a much shorter wavelength than light which reveals much more detail of the specimen, and allows useful magnification up to $\times 500\,000$
- ◆ Specimens must be completely dehydrated and mounted in a vacuum to avoid scattering of the electrons
- ◆ There are two types of electron microscope, the transmission electron microscope (TEM) and the scanning electron microscope (SEM)
- ◆ In the TEM, electrons pass through very thin sections which have been stained with salts of heavy metals such as lead and uranium to enhance differentiation between structures
- ◆ In the SEM, electrons are reflected off of the surfaces of specimens to give 3D images
- ◆ The major limitation of the use of electron microscopy is that the picture of the specimen is far removed from the reality of the living tissue or organism
- ◆ The drastic preparation of the specimen, including complete dehydration, very thin sectioning, staining, high temperatures and vacuum conditions, raises problems of the interpretation of the image, and the production of 'artificial' structures (artefacts)
- ◆ Resolution is the ability to see detail
- ◆ The higher the resolution the more of the detail present can be seen
- ◆ Magnification is enlarging in size
- ◆ Increasing magnification only reveals extra detail if the detail can be resolved
- ◆ Resolving power of light microscope limited by the wavelength of light.
- ◆ Put simply some detail is so small that it cannot be 'hit' by light, and therefore cannot be seen
- ◆ Endless magnification of the image produced by a light microscope yields no extra information
- ◆ Wavelengths of electron beams much shorter than that of light and give the electron microscope much greater resolving power than the light microscope
- ◆ Therefore extra magnification up to $\times 250\,000$ yields an equivalent amount of detail.

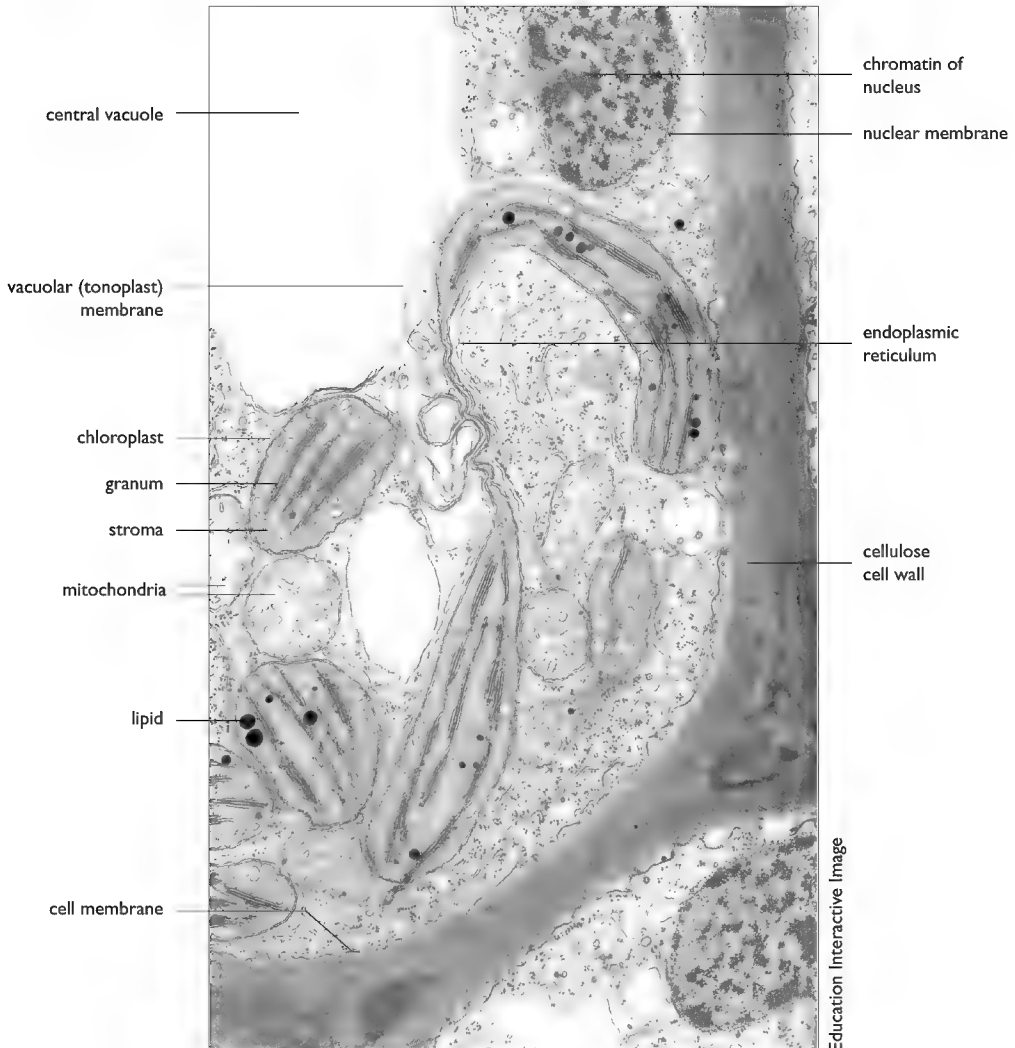
CELL ULTRASTRUCTURE

The electron microscope reveals a wealth of detail and shows the cell to be packed with a variety of structures, including **organelles**. Organelles are membrane lined compartments, found only in eukaryotic cells, and include the nucleus, mitochondria, chloroplasts and lysosomes. Each is characterised by the performance of a specific function or functions, for example, the mitochondria carry out the function of respiration. In prokaryotic cells these functions are not so localised.

EM part of an animal cell (liver)



EM part of plant leaf cell



Nucleus - the Control Centre

Even with the optical microscope, the nucleus is clearly visible, having an average diameter in animal cells (liver) of about $5\text{ }\mu\text{m}$, and in plant cells (mesophyll) of about $15\text{ }\mu\text{m}$. The nucleus stands out when cells are viewed under the microscope because of the material **chromatin**, a mixture of DNA and protein, which takes up the stains used in slide preparations. Chromatin is the material which forms the chromosomes just before nuclear division. The nucleus is surrounded by a double membrane, the **nuclear envelope** which has the same structure as, and is continuous with, the other membrane systems of the cell. Three or four thousand large pores (up to 100nm diameter) perforate the nuclear envelope, but plugs of protein and RNA appear to fill the pores.

Genes in the chromosomes direct all the cell's activities and determine its life span and reproductive cycle. The nucleus is rightly called the command centre, but it does not contain all of the genetic material of the cell. Mitochondria and chloroplasts also contain DNA and have genes of their own.

Nucleolus

This is an even more darkly staining region in the nucleus, which consists of a mass of RNA, used in the manufacture of **ribosomes**. Ribosomes are relatively small structures, about 20 nm in diameter which occur in large numbers, often several thousand per cell either bound to internal cell membranes or free in the cytoplasm. They are built in two sections called sub-units, made of approximately equal quantities of RNA and protein. They act as the sites of assembly for new proteins, manufactured both for export and for internal use.

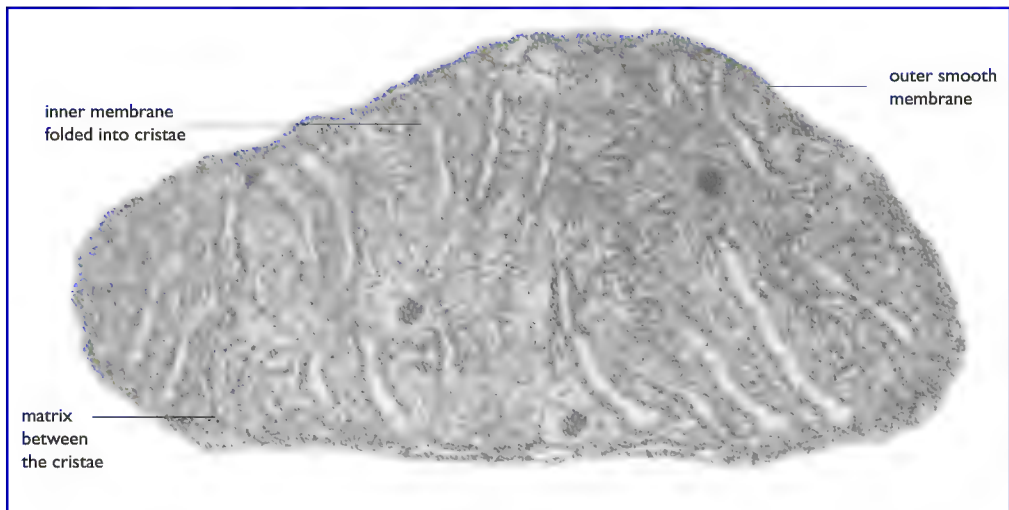
Mitochondria

Mitochondria (singular = mitochondrion) carry out aerobic respiration, which transfers energy from energy rich substances such as glucose, into the energy rich compound ATP. High energy consuming tissues like those in the muscles or brain may contain up to a thousand mitochondria in a single cell.

Mitochondria can vary greatly in length and shape, but are never greater than 1µm in diameter. There is an outer and an inner membrane. The area of the inner membrane is made as large as possible by infoldings of the membrane (**cristae**), and it is encrusted on its inner surface with spherical bodies which contain the enzymes for making ATP. The more active the cell, the more cristae and the more enzymes there are. In fact the more active the cell, the more the mitochondria increase in size and number by dividing.

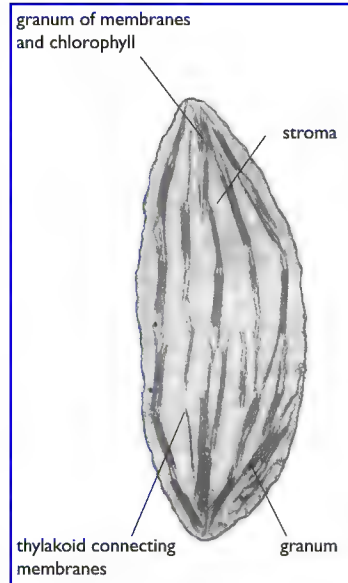
◆ CHECKPOINT SUMMARY

- ◆ The 'typical' animal cell under the electron microscope (EM) is the mammalian liver cell, as it contains a representative selection of those organelles found in animal cells
- ◆ The 'typical' plant cell under the EM is a leaf mesophyll cell
- ◆ The risk of distortion and the production of artefacts is greater with the EM as a result of the total dehydration, very thin sectioning, staining, and high temperatures generated
- ◆ It has been argued for example that the rough or granular endoplasmic reticulum is an artefact caused by the cracking and splitting of the cytoplasm pushing the ribosomes to the edge
- ◆ Mitochondria are rod-shaped organelles but appear oval in cross section. Chloroplasts are oval in structure and appear oval in cross section
- ◆ Lysosomes cannot be identified solely on appearance, the activity of digestive enzymes must be demonstrated by other means
- ◆ The rough and smooth endoplasmic reticula are not related in structure or function, and are not joined. Typically there is less smooth endoplasmic reticulum in plant cells
- ◆ Controversy surrounds the identification of centrioles in plant cells.



Chloroplasts

Chloroplasts contain the green pigment chlorophyll which absorbs light for photosynthesis. In photosynthesis light energy is used in the synthesis of organic compounds (e.g. glucose) from carbon dioxide and water. The energy is trapped in a stable form in the organic compounds. Chloroplasts, like mitochondria, have a double membrane, but there is also a third membrane system inside the chloroplast called the **thylakoid** membrane system. It is on this that the chlorophyll molecules are located. You can see from the diagram that the thylakoid membrane system is arranged in the form of stacked discs (**grana**) joined by connecting membranes (**lamellae**). The internal space is filled with a fluid (**stroma**) containing enzymes for making organic compounds, e.g. glucose and starch.



Cytoskeleton

An interconnected system of fibrous proteins gives cells their shape and a basis for movement. A number of thread like protein structures make up the cytoskeleton, notably actin, which forms thin contractile **microfilaments**; and tubulin, a protein which arranges itself into more rigid tubes resembling miniature scaffolding poles called **microtubules**. Microtubules form the supporting skeleton for the tiny cell 'hairs' called **cilia**. Microtubules are also formed and organised within areas called **centrosomes** (microtubule organising centres, or **MTOCs**) which lie very close to the nucleus. During cell division, the centrosome divides to form two new centres of microtubule formation at opposite sides of the cell. A net of microtubules (the spindle) forms between these two new centrosomes on which chromosomes are held and pulled apart.

In animal cells (but not all plant cells) the centrosome contains two structures, which can be seen in electron micrographs lying at right angles to each other. These are called **centrioles**. Centrioles also form the basal bodies at the base of cilia.

Plasma (cell surface) membrane

This controls the entry and exit of all materials into and out of cells. The electron microscope does not reveal any details of its structure, which is modelled on evidence of biochemical investigations. It is fatty in nature and contains proteins and other substances. It is partially permeable, being more permeable to water, than to dissolved solutes which pass through at different rates depending on a complex of factors.

In cells which are specialised for the exchange of materials across the plasma membrane e.g. cells lining the small intestine and parts of the kidney tubules, the surface area may be dramatically increased by microvilli. Microvilli are extensions of the plasma membrane which protrude out of the surface like the bristles of a hair brush.

Rough and smooth endoplasmic reticulum

In addition to the outer surface (plasma) membrane and those which surround the organelles, cells (especially secretory cells), have extensive internal membrane systems, known as the **endoplasmic reticulum** (ER). The ER is a maze of membrane bound tubules and

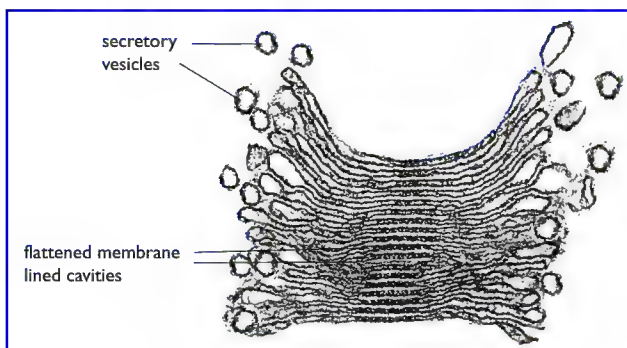
layers of flattened sacs enclosing a fluid filled interior, which acts as an intracellular (inside the cell) transport and storage system. It is continuous with the nuclear membrane and similar in structure.

The rough ER has ribosomes associated with it on the cytoplasmic side of the membranes. The ribosomes are the sites of protein synthesis.

The smooth ER tends to be more tubular, and appears to have a complex set of functions, including fat metabolism. The two types of ER are not connected.

Golgi apparatus

This is more clearly seen in animal cells and is a region of stacked smooth ER specialised for the purpose of collecting, processing and redirecting proteins and fats made in the rough ER. Materials are transported between the ER, the Golgi apparatus, and the plasma membrane, sealed in membrane sacs called vesicles which bud off from one membrane, and then fuse with another. Substances are taken in (**endocytosis**) and expelled (**exocytosis**) by the plasma membrane in this way. For example, digestive enzymes produced by cells of the pancreas are secreted by exocytosis into the pancreatic duct.



Lysosomes

Vesicles produced by the Golgi apparatus are specialised for particular functions. Animal cells, have lysosomes which contain a cocktail of digesting enzymes. They break down and dispose of unwanted materials or damaged structures in the cell, expelling waste debris by exocytosis, and recycling reusable molecules within the cell. They also meet and fuse with vesicles carrying imported food substances, or immobilised bacteria, from the cell surface, and digest the contents.

It is clear from this that the membranes surrounding lysosomes must act as impermeable barriers preventing the leakage of its digestive enzymes into the cytoplasm, this process (**autodigestion**) occurs after the death of a cell and plays an important part in its removal.

◆ CHECKPOINT SUMMARY

- ◆ Functions of organelles determined by differential centrifugation, and biochemical investigation of samples from each 'organelle band'
- ◆ Centrifuged 'organelle bands' not completely 'pure'
- ◆ Lysosomes poorly identifiable in electron micrographs, identification almost entirely on functional criteria
- ◆ Radioactive tracers can locate functions in specific organelles
- ◆ Cells specialised for particular functions show organelles developed in similar way, e.g. active voluntary muscle, and secretory cells, have an increased number of larger mitochondria.
- ◆ Smooth endoplasmic reticulum associated with a wide range of metabolic functions suggesting that its identification as a specific organelle is unlikely
- ◆ Existence of many ribosomes arranged in chains (polyribosomes) suggests protein synthesis (translation of mRNA) in action
- ◆ There are still major problems in correlating specific functions with clearly identifiable organelles
- ◆ Function of chloroplasts easily established with the use of the light microscope, i.e. recognising cell fraction that carries out photosynthesis.

The principal features of a bacterium

The features and organelles described above are typical of eukaryotic cells. Bacterial cells are **prokaryotic**. Their structure varies greatly to match the diversity of life style and habitat (see 10.1) and the illustration here is designed to show the major features which may be present, in a diagrammatic, generalised form. (Remember, no individual bacterium actually looks like this.)

Compare and contrast this structure with that of the eukaryotic cells. Most obviously, there are no large membrane-bound organelles. Instead of mitochondria and chloroplasts, the enzymes for respiration (and photosynthesis where this occurs) are located on simple membrane systems. In the absence of a nucleus, the DNA is formed, with its associated proteins, into a single main coil, the bacterial chromosome. Additional small loops of DNA called plasmids may also occur. Not all prokaryotes are able to move, but some possess flagella, which are like elongated cilia but without the microtubules, capable of propelling the organism through water. The cell wall is formed from a mixture of polysaccharides and amino acids called murein (not cellulose as in plants). Many bacteria which cause disease (pathogenic bacteria) have a slime capsule as their outermost layer which helps in protection against the body defences of the infected organism.

◆ CHECKPOINT SUMMARY

- ◆ Prokaryotic cells (bacteria) lack a true nucleus and membrane bound organelles
- ◆ The DNA is located in a central strand, and as circular plasmids in the rest of the cytoplasm
- ◆ The cell wall is not cellulose as in plant cells
- ◆ Respiration is located in mesosomes which are infoldings of the cell surface membrane, sharing the principle of increased surface area of membranes with true mitochondria
- ◆ The origin of mitochondria and chloroplasts as mutualistic prokaryotes with eukaryote cells, explains their absence in prokaryotes
- ◆ Ribosomes are present in both prokaryotes and eukaryotes
- ◆ Prokaryotic cells may form colonies but never multicellular organisms/tissues.

CELL FRACTIONATION

Ultracentrifugation to separate cell components

The function of individual organelles has been established largely by isolating each organelle from the rest of the cell components, and subjecting it to conditions which copy those which are thought to exist in the natural state. Naturally, the most detailed functional information has resulted from the organelles which are most easily isolated, namely mitochondria and chloroplasts. The isolation of subcellular components starts with a mechanical disruption of the cells to produce subcellular fragments, typically by breaking up fresh tissue in a test tube homogeniser. A pH buffer and sucrose solution are added to keep the cell fragments at optimum pH and ensure that the water potential of the surrounding liquid remains the same as organelles. Enzyme action and bacterial activity is slowed down by immersing tubes containing cell fragments in ice.

The fragments are separated by a series of spins in a high speed centrifuge (ultracentrifuge). The heaviest components (fractions) separate at the lowest spinning speeds. At speeds 1000 times the pull of gravity, the cell nuclei separate out into a pellet at the bottom of the tube. The remaining liquid (supernatant), containing all the other cell fragments may then be transferred to another tube and recentrifuged at a higher speed. Mitochondria form a pellet at about 3000 times gravitational pull and the smaller cell fragments require even greater speeds. Isolated fragments are never pure, particularly the smaller components such as lysosomes because the cell membrane systems (ER, Golgi apparatus, nuclear and plasma membranes) reform into small vesicles of similar size when cells are broken up. Isolated cell fragments may be purified further by centrifugation in tubes which are prepared with concentrated gel-like solutes in the form of a density gradient so that the solution at the top of the tube is less dense than that at the bottom. As the tube is spun with the selected sample, so the cell fragments separate in bands at a level in the tube corresponding to their own density.

◆ CHECKPOINT SUMMARY

- ◆ Isolation of subcellular components starts with a mechanical disruption of the cells to produce subcellular fragments
- ◆ A pH buffer and sucrose solution is added to prevent disruption of the organelles, and the temperature is lowered to prevent autodigestion by enzymes, and decomposition by bacteria
- ◆ The suspension of cell fragments is spun at high speeds in an ultracentrifuge
- ◆ The force that is exerted upon the suspension forces the organelles downwards to the bottom of the tube according to their mass and density
- ◆ The nuclei are the first to be forced down at about 1000G (1000 x the force of gravity)
- ◆ The supernatant liquid can be separated off and respun at higher speeds
- ◆ Mitochondria spin down at about 3000G.
- ◆ Smaller organelles like the ribosomes require much higher forces to separate them out
- ◆ Isolated samples are never pure, and can be further purified by recentrifuging in a liquid medium of graded density, with the greatest density at the bottom
- ◆ Organelles separate at a level in the liquid corresponding to their own density.

1.3

The Properties of plasma membranes related to the passage of substances through them

Contents

Plasma membranes

- ▼ The arrangement of phospholipids, proteins and carbohydrates in the fluid-mosaic model of membrane structure

Diffusion

- ▼ Diffusion and the factors which determine its rate. Fick's law

Water potential

- ▼ Osmosis - the movement of water from a solution of less negative water pressure to a solution of more negative water pressure through a partially permeable membrane

Active transport and facilitated diffusion

- ▼ The role of protein molecules in active transport and facilitated diffusion
- ▼ Endocytosis and exocytosis

PLASMA MEMBRANES

Introduction

The cell surface membrane (**plasmalemma** or **plasma membrane**) and the membranes which enclose the cell organelles and make up the extensive system of interrelated channels and vesicles within the cell share the same structure. It is a structure which is so flexible that it can break and reform easily yet it is capable of performing complex functions; acting as a barrier, container, transport regulator and as a site of recognition, distinguishing between 'friendly' and 'foreign' substances, 'self' and 'non-self'.

Membrane Structure - the fluid-mosaic model

Membranes are constructed from lipid, protein and carbohydrate components. The structure is based on a bilayer of phospholipid molecules, strengthened by cholesterol. Phospholipids adopt this bilayer structure automatically in fluid surroundings because the fatty acid 'tails' of the molecules are water repelling (hydrophobic) whilst the phosphate/alcohol 'heads' are water attracting (hydrophilic).

Proteins of various sizes and shapes are located on and in the bilayer. Some are bonded to the hydrophilic head region and do not penetrate the interior of the membrane (**extrinsic proteins**), others, have one end buried within the fatty layer and one end sticking

outside of the cell membrane (**intrinsic proteins**). A third kind, (**transmembrane proteins**), pass all the way through the membrane to create special channels. The carbohydrate component of cell membranes consists of short polysaccharide chains joined to membrane proteins or fats forming **glycoproteins** and **glycolipids** respectively, which project outside the cell membrane.

Individual molecules of phospholipid and protein in the membrane have a degree of **mobility** within the membrane. This fluidity, coupled with the bubbled appearance of the proteins on the surface of membranes gave rise to the the term '**fluid mosaic model**' when the structure was first proposed. Cholesterol stabilises membranes by limiting the movement of phospholipid molecules within the membrane.

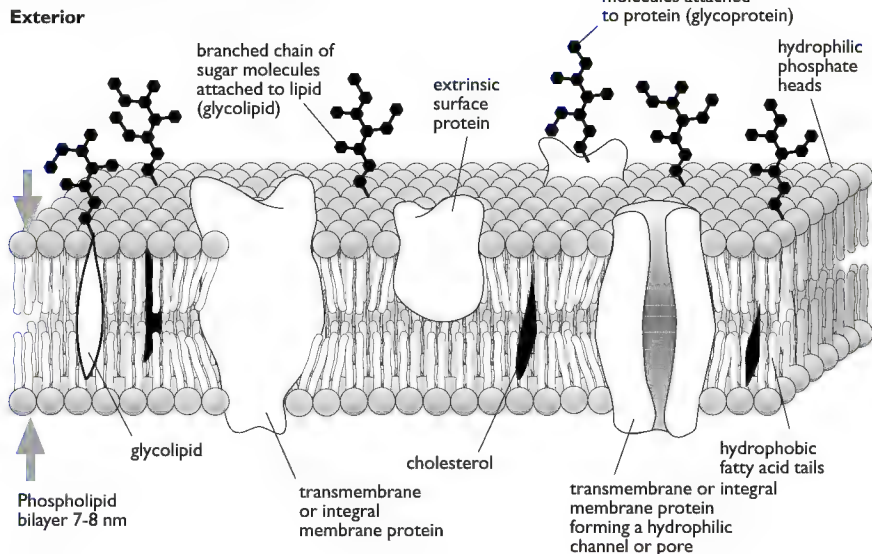
Cells have the ability to sense and respond to the presence of a great variety of molecules. This ability is due to proteins in the surface membrane which act as **receptors**. Hormones such as insulin and adrenaline, and many drugs and toxins lock onto recognition sites on receptor proteins triggering changes in the cell's behaviour.

Glycoproteins and glycolipids have a very important role in personalising a cell's identity. They are arranged in the membrane bilayer with the chain of sugar molecules to the outside. The possibility for variation in the structure and different combinations of these chains gives an endless range of different surface patterns, and forms the basis of a recognition system. Each individual has his or her own cell surface 'print'. 'Self' recognises 'self', and immune systems are mobilised when 'non-self' substances are encountered. The glycoproteins and glycolipids of the cell membrane are responsible for an individual's tissue type, a well known example of which is the blood groups.

◆ CHECKPOINT SUMMARY

- ◆ All cell membranes are thought to share the same structure except the inner membrane of mitochondria
- ◆ This is based on a lipid bilayer formed from phospholipids aligning themselves with their hydrophobic poles next to each other within the membrane, and hydrophilic poles facing outwards
- ◆ This fluid arrangement is stabilised by the presence of another lipid - cholesterol
- ◆ Various proteins are embedded within this lipid bilayer
- ◆ Extrinsic proteins are found on the surface of the membrane
- ◆ Intrinsic proteins have one end embedded in the lipid bilayer and the other protruding out of the external surface of the membrane
- ◆ Transmembrane proteins span the lipid bilayer and create aqueous channels of pores through which water soluble substances can pass by diffusion.
- ◆ Short polysaccharide chains attached to proteins (glycoproteins) and to fats (glycolipids) protrude from the external surface and act as cell recognition sites (receptors or antigens)
- ◆ The patchwork arrangement of all these types of molecules in the lipid bilayer is the origin of the description 'mosaic'.

Fluid mosaic model of cell surface membrane



Transport across cell membranes

The plasma membrane acts as a partially permeable barrier to the passage of substances into and out of the cell, for example water and fat soluble substances pass through more easily than sucrose. The different rate of passage of electrically charged ions generates membrane potentials which in turn influence the further uptake of charged ions, and are also involved in cell sensitivity, particularly in nerve cells.

The transport of respiratory gases, nutrients and metabolic waste products occurs through the membrane, either directly through the bilayer, or via channels or 'pores' created by transmembrane proteins. In some cases the substances move down a concentration gradient (i.e. from higher concentration to lower concentration) which is known as **passive transport**. In others, substances are moved against the concentration gradient (i.e. from lower concentration to higher concentration) in a process requiring energy in the form of ATP, which is known as **active transport**.

DIFFUSION and the factors which determine its rate

Molecules and ions may move passively by diffusion, which is defined as the overall (net) movement of substances from regions of their higher concentration (higher chemical potential) to regions of their lower concentration (lower chemical potential) until equilibrium is reached. The movement of the particles of substances in diffusion is random in all directions, as a result of their inherent kinetic energy. If there are more in one area than another, the net effect is for the particles to distribute themselves equally throughout that particular space, i.e. the concentration gradient evens out until at equilibrium there are an equal number of particles moving in all directions.

The speed of these diffusing particles is only increased by a rise in temperature, which increases their kinetic energy. In addition to the effect of temperature on diffusion, the amount of a substance that diffuses in a given time (rate of diffusion) is dependent upon the difference in its concentration in two separated regions (the **concentration gradient**), the surface area of the separating boundary or exchange surface, and the distance separating them i.e. the thickness of the exchange surface; a relationship expressed in the following equation known as **Fick's law**.

$$\text{Rate of diffusion} = \frac{\text{difference in concentration} \times \text{surface area}}{\text{thickness of the exchange surface}}$$

This gives a rough guide to the rate at which a substance such as oxygen diffuses from the air in an air sac (alveolus) in the lung into the blood in a lung capillary. In principle, the greater the surface area, the greater the concentration difference, and the thinner the separating layers, the greater the rate of diffusion.

The most important factor affecting diffusion across cell membranes, however, is the chemical nature of the substance being transported. Diffusion across the bilayer depends principally upon the solubility of the substance in fat. You will remember that the phospholipid bilayer is strongly hydrophobic. Polar molecules such as glucose, some amino acids, and inorganic ions pass through very slowly or not at all, whilst non polar molecules such as calciferol (vitamin D) move across with ease. It is interesting to note that the first anaesthetics (chloroform and ether) were fat solvents that disrupted membrane structure and function especially in nerve cells.

◆ CHECKPOINT SUMMARY

- ◆ The cell surface membrane defines the limits of the cell
- ◆ It receives 'messages' by providing receptor sites for hormones, cell recognition of 'self' and 'non-self', and act as antigens with which antibodies can bind
- ◆ It acts as a partially permeable membrane regulating the transport of substances across the membrane
- ◆ Internal membrane systems provide a surface for attachment of organelles and enzymes, and compartmentalise specialised functions within the cell.
- ◆ Passive diffusion occurs across membranes down diffusion gradients from high concentration to low concentration. Fat soluble substances diffuse faster than non-fat soluble substances which must pass through the aqueous pores
- ◆ The speed of these diffusing particles is only increased by an increase in temperature, which increases their kinetic energy. In addition to the effect of temperature on diffusion, the amount of a substance that diffuses in a given time (rate of diffusion) is dependent upon the difference in its concentration in two separated regions (the concentration gradient), the surface area of the separating boundary or exchange surface, and the distance separating them i.e. the thickness of the exchange surface; a relationship expressed by Fick's law: Rate of diffusion is proportional to the difference in concentration x surface area: divided by the thickness of the exchange surface.

WATER POTENTIAL

Osmosis

Cell membranes are **partially permeable**, allowing the passage of substances at different rates. Generally cell membranes are more permeable to water than to many solutes.

Water moves according to the same principles of diffusion, but it is not usual to refer to high and low 'concentrations' of water - the term 'concentration' being traditionally associated with any solutes dissolved **in** water in a solution. Therefore the preferred term is the chemical potential of water or **water potential**.

Water always moves (diffuses) from a region of higher water potential to one of lower water potential. You could think of water potential as the **tendency for water molecules to move** i.e. diffuse. The higher the water potential the more easily will the water molecules move, and the lower the water potential the less easily will the water molecules move.

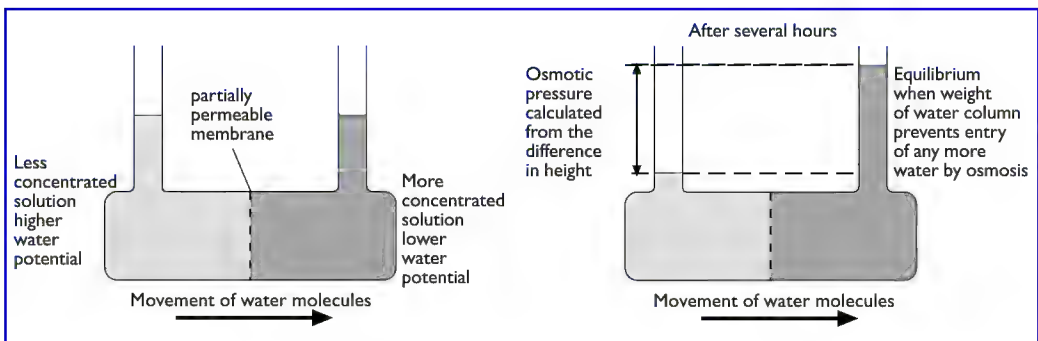
At standard pressure and temperature, pure water has the highest water potential. If substances are dissolved in solution then the water potential is decreased because the solutes associate with water to a greater or lesser extent and reduce the ease with which the water molecules can move. Therefore any solution has a lower water potential than pure water, and a more concentrated solution has a lower water potential than a less concentrated solution.

Water potential of a fluid can be described as the tendency of water to move into it from pure water. If the fluid **is** pure water, there is no movement, so the **water potential of pure water is zero**. The water potential of any solution is less than pure water and is therefore negative. The more negative the water potential the greater the tendency of water to move into that system.

Where a solution is separated by a partially permeable membrane from pure water or a less concentrated solution, water will diffuse into the more concentrated solution until equilibrium is reached. The special case of the diffusion of water across a partially permeable membrane is known as **osmosis**.

◆ CHECKPOINT SUMMARY

- ◆ Osmosis is the special case of the diffusion of water from regions of higher water potential to regions of lower water potential across a partially permeable membrane which is more permeable to water than to dissolved solutes. (If the membrane was fully permeable the solutes would diffuse down their diffusion gradient and the water would not move)
- ◆ At room temperature and pressure pure water has the highest water potential which is given the value of zero. Solutions have a water potential of less than zero (negative)
- ◆ Facilitated diffusion occurs via specialised carriers in the membranes which reduce the resistance to the diffusion of these substances
- ◆ Active transport involves energy in the form of ATP from respiration to pump ions across the cell surface membrane via carriers against their diffusion gradient
- ◆ Mass or bulk transport of fluids into and out of the cell is achieved by vacuoles fusing with, or being pinched off from, the cell surface membrane respectively.



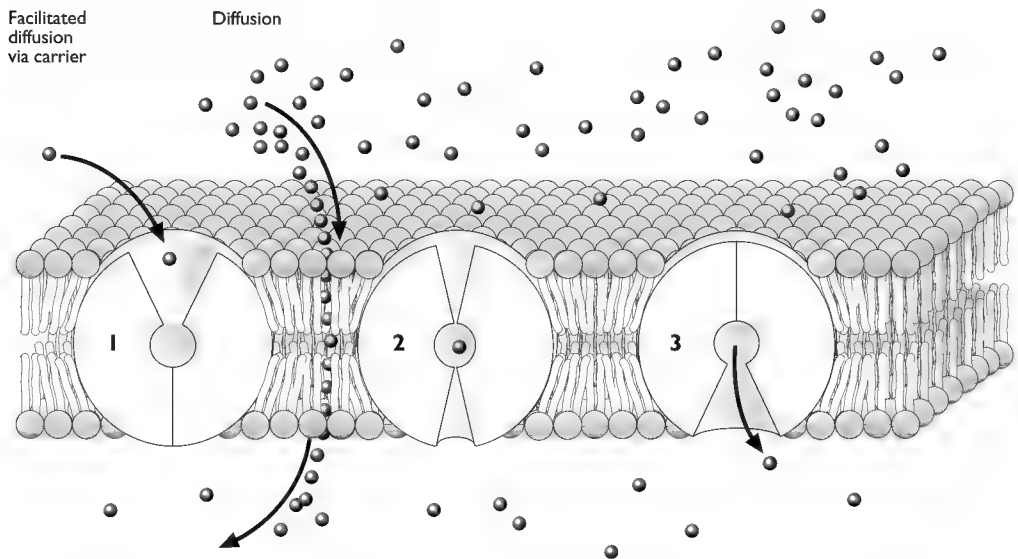
FACILITATED DIFFUSION

The transport of polar molecules e.g. glucose, some amino acids, and inorganic ions, across cell membranes, occurs through hydrophilic channels formed by transmembrane proteins, in a process called **facilitated diffusion**. Polar molecules also become temporarily attached to special binding sites on transmembrane proteins called **carriers**. These are rather like enzymes in that they are shape specific and accelerate a process which is already 'downhill' in terms of energy. All that is needed is a concentration gradient. The attachment of a molecule to the binding site alters the overall shape of the carrier in such a way as to deposit the molecule on the other side of the membrane along their normal concentration gradients.

As has been seen, the transport of inorganic ions such as sodium (Na^+), potassium (K^+), calcium (Ca^{++}) and chloride (Cl^-) across cell membranes depends upon transmembrane proteins.

Some transmembrane proteins form selective **ion channels** through which the facilitated diffusion of ions occurs down their concentration gradients. These ion channels cannot be coupled to an energy source, and cannot be involved in active transport across the membrane.

Diagram to show diffusion and facilitated diffusion



ACTIVE TRANSPORT

Some transmembrane proteins act as active **ion pumps**, which use energy (in the form of ATP) from respiration, to move ions against their concentration gradients. The ion channels can open and close like 'gates', and the activity of the ion 'pumps' can be varied. In these ways, the membrane can be made selectively permeable to certain ions at certain times.

As a result of the distribution of ions on either side of the membrane **transmembrane potentials** are created, in which there is an overall difference in electric charge between the inside and the outside of the membrane. In normal conditions, the inside of the cell membrane is negatively charged relative to the outside, and this will assist the uptake of positively charged ions (cations) and oppose the uptake of negatively charged ions (anions). Thus the movement of ions across membranes is influenced by both electrical and chemical gradients i.e. **electro-chemical gradients** (these have particular importance for the function of nerve and muscle cells).

Some of these transmembrane protein carriers carry two substances at once in a **coupled mechanism**. A common example is the sodium/potassium pump (Na^+/K^+ pump), in which the active pumping of sodium out of a cell is coupled with the pumping-in of potassium, both against their diffusion gradients (for every two sodium ions pumped out, three potassium ions are pumped in, resulting in high potassium and low sodium levels within the cell). Another example is where the sodium pump is linked to the uptake of glucose. The sodium ions are pumped actively out of the cell and diffuse back into the cell via a carrier associated with glucose. In this case the glucose is moving passively down its concentration gradient, but at a faster rate as a result of its association with the re-entry of sodium which is kept at a high rate by its being continually pumped out of the cell.

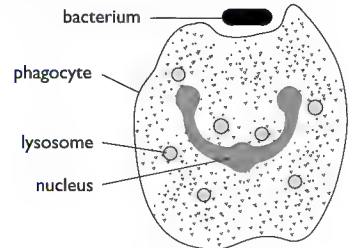
Endo and exocytosis

The bulk transport of liquids and solids is achieved by membranes pinching off small bubble-like vesicles which can fuse with other membranes to transfer their contents. Uptake of substances into the cell in this way is known as **endocytosis**, and elimination of substances as **exocytosis**.

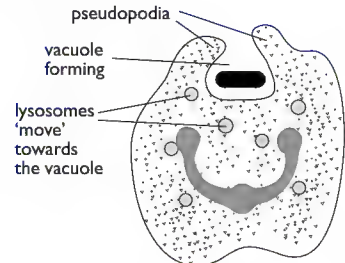
The term **phagocytosis** is used to describe the kind of endocytosis in which relatively large solid particles are 'ingested' by a cell, as in the case of white blood cells which engulf bacteria or *Amoeba* taking in food. In this process the outer membrane folds inwards to completely surround the ingested particle. It is then transported into the interior of the cell within a sealed vesicle which functions as a temporary digestive chamber and, in *Amoeba*, is termed a food vacuole. Lysosomes containing digestive enzymes fuse with and release their contents into the food vacuole and the soluble products of digestion are transported out for assimilation by the cell. Fluids may be taken up in the same way. This process is sometimes referred to as **pinocytosis**.

Undigested matter may be expelled from the cell by the reverse process (exocytosis), similarly fluid secretions produced by cells are transported to the plasma membrane in small vesicles, which fuse with it and release their contents.

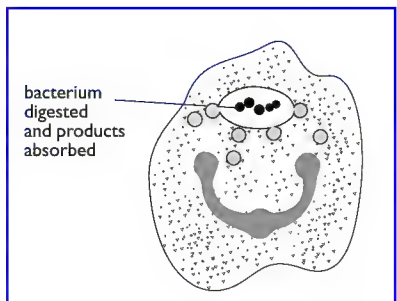
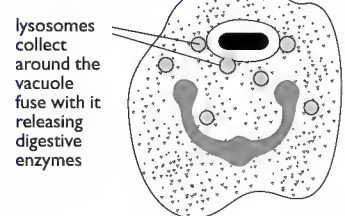
Phagocyte 'moves' towards bacterium



Vacuole forming



Vacuole formed



1.4

Large molecules in the structure and functioning of cells

Contents

Biological molecules

- ▼ Monomers and polymers.
- ▼ Condensation and hydrolysis

Carbohydrates

- ▼ Structure and properties of carbohydrates.
- ▼ The structure of alpha and beta glucose, and the linking of these monomers by glycosidic bonds
- ▼ Biochemical tests for reducing sugars, non-reducing sugars, starch
- ▼ The basic structure and function of starch, glycogen and cellulose

Proteins

- ▼ Amino acid structure and the peptide bond
- ▼ Primary, secondary, tertiary and quaternary structure of proteins
- ▼ The relationship of tertiary structure to function in globular proteins
- ▼ The biuret test for proteins

Lipids

- ▼ The structure of saturated and unsaturated triglycerides and phospholipids
- ▼ The emulsion test for lipids

Chromatography

- ▼ The technique of chromatography for separating and identifying molecules
- ▼ The calculation and use of R_f value
- ▼ Two-way chromatography

BIOLOGICAL MOLECULES

The bodies of living organisms are made up of fewer than 20 different chemical elements and just six of these bioelements make up 99% of the total body mass (oxygen 65%, carbon 18%, hydrogen 10%, nitrogen 3%, calcium 2%, phosphorus 1%). Most of the important biological molecules are organic, which means that they are made up from carbon compounds (note that most but not all compounds containing carbon

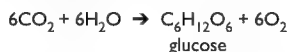
are organic; exceptions include carbon dioxide and carbonates). Organic biomolecules include carbohydrates, proteins, lipids and nucleic acids. Individual molecules can act as sub-units (**monomers**) to combine by reactions called **condensation reactions**, in which a molecule of water is lost as each sub-unit joins the next. In this way monomers can form dimers (two joined monomers e.g. glucose and fructose forming sucrose), or chains of repeating sub-units (**polymers**). Bonds formed by condensation may be broken down by the reverse process, **hydrolysis**, which involves the addition of a molecule of water for each bond broken. The following account describes the structure of carbohydrates, proteins and lipids and the relationship of molecular structure to their function in living organisms.

CARBOHYDRATES

Structure and properties of carbohydrates

The structures of alpha and beta glucose

Carbohydrates are a family of molecules made up, as the name suggests, from carbon and the components of water (hydrogen and oxygen). Carbohydrates are manufactured by plants and are passed on to other organisms via food chains. You should already be familiar with the name and chemical formula of one of the simpler carbohydrate molecules, glucose, which is made from CO_2 and H_2O in the process of photosynthesis.

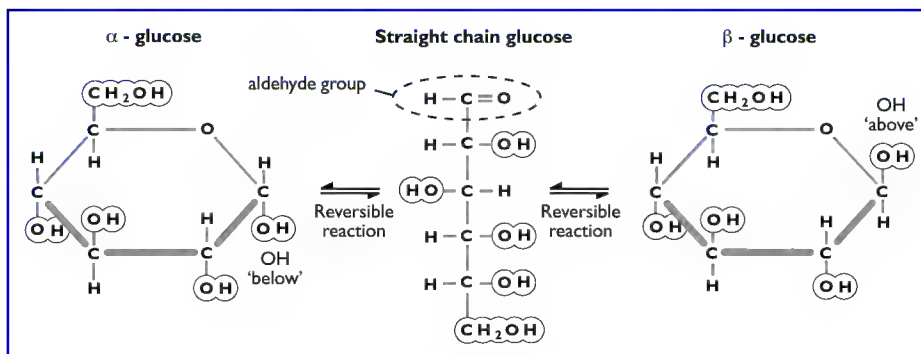


Glucose has six carbon atoms in the form of a framework to which the oxygen and hydrogen atoms are attached. In dry glucose powder the carbon atoms are arranged in a straight chain, but if it is dissolved in water, the chains form themselves into ring structures. Two different ring forms exist called **alpha glucose** and **beta glucose**. The carbon atoms are numbered 1 to 6 starting in a clockwise direction from the position of the oxygen atom. As discussed later, the different structures of alpha and beta glucose result in the formation of polysaccharides with different properties.

Alpha and beta glucose are **isomers**; they have the same chemical formula but a slightly different arrangement of atoms in the molecule (fructose and galactose are also isomers of glucose).

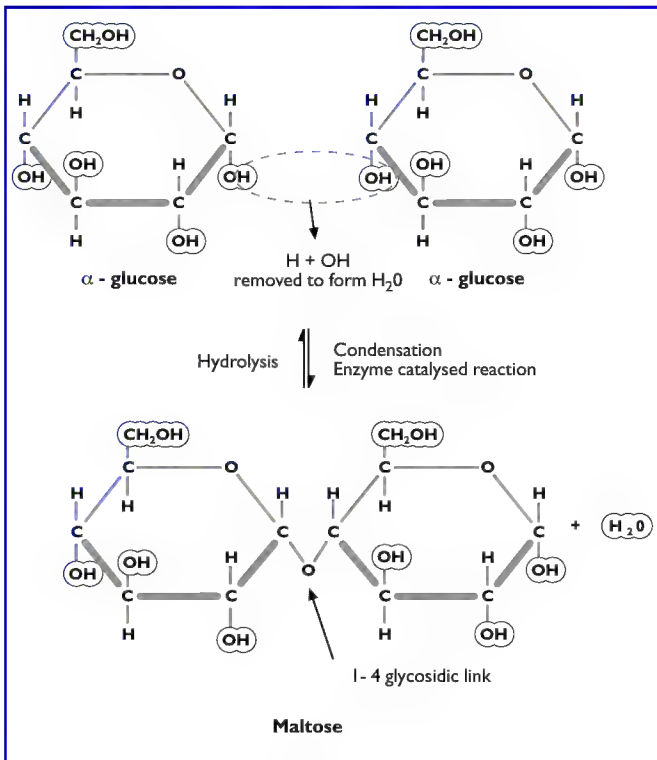
◆ CHECKPOINT SUMMARY

- ◆ Most of the important biological molecules are 'organic', which means that they are made up from carbon compounds
- ◆ Not all compounds containing carbon are organic. Exceptions include carbon dioxide and carbonates)
- ◆ Organic biomolecules include carbohydrates, proteins, lipids and nucleic acids
- ◆ Individual molecules (monomers) can join to form dimers of just two monomers, or longer chains (polymers) of repeating monomers.
- ◆ Polymers are very large molecules, built up by reactions called condensation reactions in which a molecule of water is lost as each sub-unit joins the next
- ◆ Bonds formed by condensation reactions may be broken down by the reverse process, hydrolysis, which involves the addition of a molecule of water for each bond broken
- ◆ Condensation and hydrolysis reactions are catalysed by enzymes in the living cell
- ◆ Under laboratory conditions, in the absence of a catalyst, hydrolysis requires extreme conditions of temperature and pH.



The formation and breakage of glycosidic bonds

Ring structures like these are able to link together to form pairs or long chain molecules. The terms monosaccharide, disaccharide and polysaccharide are used to describe the number of glucose molecules making up a chain (mono = 1, di = 2, and poly = many). If the units of glucose link up to form larger molecules, the first carbon atom of one alpha glucose molecule links to the fourth carbon atom of another with the elimination of a molecule of water. This enzyme controlled reaction results in an oxygen bridge called a **glycosidic bond** (an alpha 1-4 glycosidic bond) and in the formation of the disaccharide **maltose**. The formation of more than one bond by condensation leads to longer and longer 'multi-molecules' or polymers.



Test for reducing sugars e.g. glucose

All the common simple carbohydrate sugars, with the notable exception of sucrose (table sugar), have the ability to **reduce** other chemicals. They are thus called reducing sugars and they can be identified by heating with **Benedict's reagent**. Benedict's reagent contains copper sulphate and has a clear blue colour. This changes to brick red as the copper is reduced to a solid precipitate of copper oxide in the presence of a reducing sugar. The quantity of reducing sugar in a positive test can be estimated from the amount of precipitate formed using a colorimeter or a standard colour comparison sequence.

Test for non-reducing sugars

Sucrose gives a negative result with the Benedict's test, but when heated to boiling with dilute hydrochloric acid it is hydrolysed into its constituent monosaccharides e.g. glucose and fructose, which when made alkaline and retested with Benedict's reagent give a positive result.

If a mixture of both reducing and non-reducing sugars are present, which would be highly likely with plant tissue extracts, the precipitate from the initial positive Benedict's test would need to be removed by filtration, and the resultant clear filtrate re-tested for sucrose as described above.

Structure and function of starch, glycogen and cellulose

Glucose is the universal fuel for respiration, but it cannot be stored in cells because a high glucose concentration would result in the entry of large amounts of water by osmosis which could cause cells unprotected by a cell wall or surrounding cells, to swell up and burst. For this reason, glucose in excess of energy requirements is converted for storage to the relatively insoluble polysaccharides **starch** in plant cells and **glycogen** in animal cells. These substances are similar in chemical structure, both being formed from chains of alpha glucose molecules.

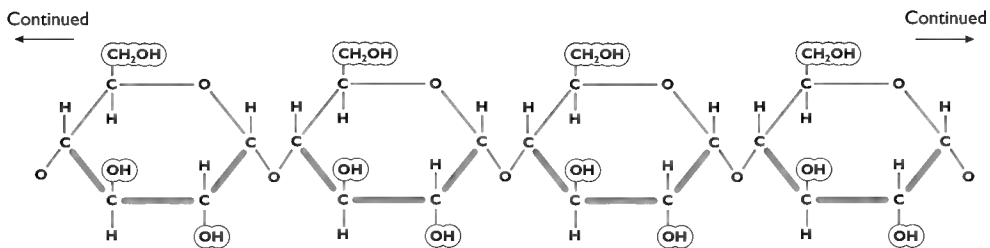
Starch is a mixture of **amylose** (unbranched chains of alpha glucose) and **amylopectin** (branched chains of alpha glucose). When combined together, the alpha glucose units have their oxygen bridges on the same side of the chain. Hydrogen bonds form between them and cause the chain to coil into a helix, so starch molecules have a dense, tightly packed structure and are relatively insoluble in water.

To dissolve starch, it is necessary to heat it. This breaks the hydrogen bonds and, as the coils begin to open, water molecules rush to occupy the exposed polar sites binding all over the surface. This is why starch suddenly swells in hot water and is the secret of starch based sauce thickeners used in cooking.

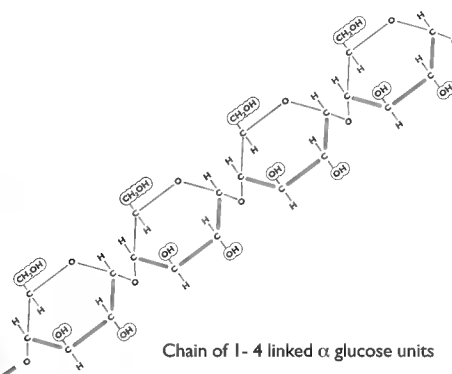
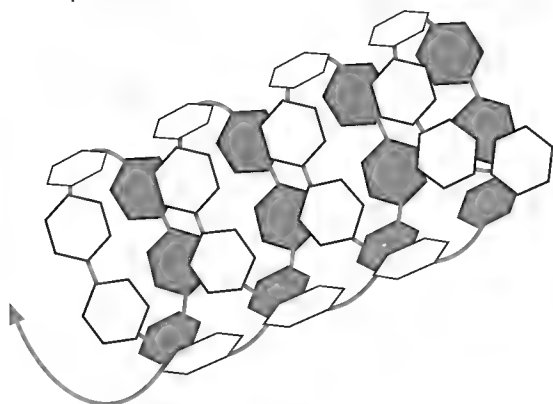
Test for Starch

A solution of iodine in potassium iodide is added to a solution of the crushed tissue at room temperature. The presence of starch is indicated by a colour change from light brown to blue-black.

Part of amylose molecule chain

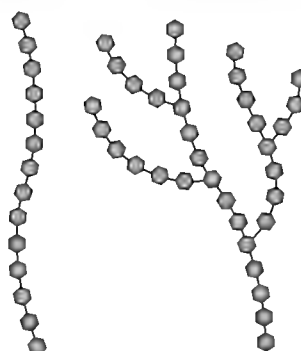


Hydrogen bonds between CH_2OH groups which are all on one side 'pull and twist' the chain to form a helix



Amylose - straight chains of glucose molecules

Amylopectin - branched chains of glucose molecules



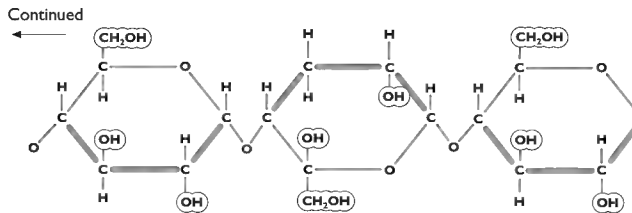
Glycogen has a similar structure to amylopectin but has a large number of shorter branches. It is more suited to animal metabolism as it is capable of being broken down more rapidly than starch.

Cellulose is the major component of plant cell walls. The characteristic fibrous structure of cellulose is created by chains of 1-4 linked beta glucose molecules. The beta molecules, when joined together, have their oxygen bridges on alternate sides of the chain, and thus it cannot form into a helix, but by forming hydrogen bonds with other chains lying side by side they make flat ribbons, which in turn form fibres.

The basic meshwork of cellulose fibres in plant cell walls is strengthened and cemented by other materials such as pectin and lignin. Cellulose is the main component of numerous textiles e.g. cotton, linen, hemp; timber products, paper and plastics e.g. celluloid.

Starch and cellulose have very different properties which arise as a result of one difference between alpha and beta glucose. Starch has no distinct structure (amorphous), no structural strength, is slightly soluble in water, and digestible by vertebrates. Cellulose has a distinct fibrous structure, great structural (tensile) strength, is insoluble in water and indigestible by vertebrates. (Herbivorous vertebrates harbour mutualistic microorganisms in specialised compartments of their gut which secrete cellulase enzyme which digests cellulose.)

Part of a cellulose molecule chain



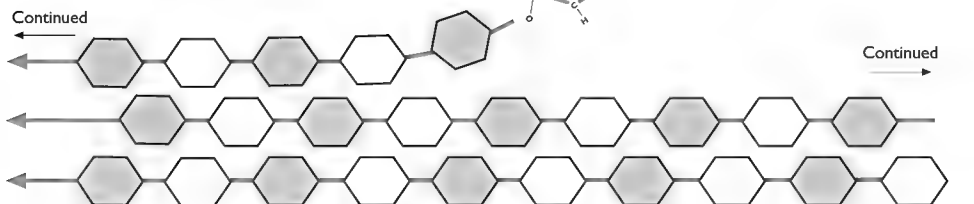
Notice how the CH_2OH groups alternate from side to side of the chain (the bonds indicated by a thick line are nearer)

◆ CHECKPOINT SUMMARY

- ◆ Simple formula for glucose - $\text{C}_6\text{H}_{12}\text{O}_6$
- ◆ Forms straight chain in dry powder form
- ◆ Forms ring structure in solution
- ◆ alpha and beta glucose are isomers differing in the 3D arrangement of their atoms
- ◆ Monosaccharides (glucose, fructose and galactose) combine together with the elimination of water to form glycosidic bonds - a condensation reaction.
- ◆ Glycosidic bonds are broken by the addition of the elements of water across the bond - a hydrolysis reaction
- ◆ Starch is a mixture of amylose (unbranched chains) and amylopectin (branched chains).
- ◆ Both being long chains (polymers) of alpha glucose molecules
- ◆ When combined the alpha glucose molecules have their oxygen bridges on the same side of the chain, hydrogen bonds form between them and cause the chain to coil into a helix
- ◆ Starch molecules are relatively insoluble in water
- ◆ Cellulose is a polymer of beta glucose which have their oxygen bridges on alternate sides of the chain and so cannot form a helix
- ◆ Instead they form hydrogen bonds with other adjacent chains to make flat ribbons which form cellulose fibres.

Arrangement of molecules in microfibril

Chains of 1-4 linked β glucose units lie parallel forming a microfibril



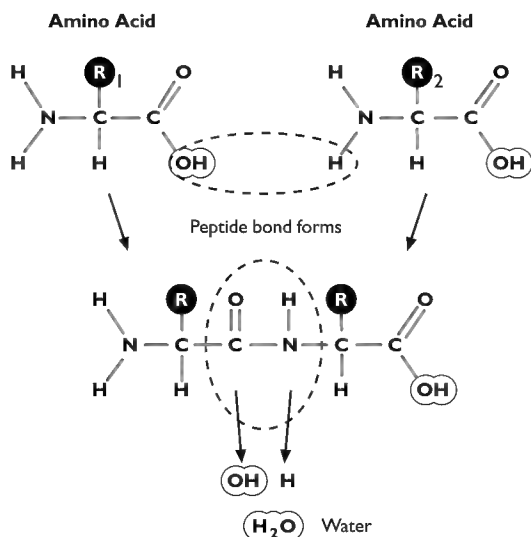
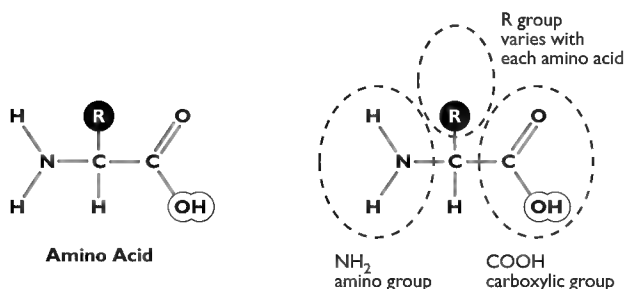
Hydrogen bonds form between alternating CH_2OH units bind molecules together side by side into microfibrils.

PROTEINS

Proteins are polymers made up of **amino acids**, joined together in long chains, which may be folded and twisted in various ways. There are as many as 100 000 different kinds of protein molecule in human cells. This variety of structure is made possible by the fact that the twenty different types of amino acid commonly found in proteins can be arranged in any order in chains up to 2000 units long.

Amino acids and peptide bonds

The chemical structure of an amino acid consists of a central carbon atom bonded to a hydrogen atom, a carboxyl group ($-\text{COOH}$), an amino group ($-\text{NH}_2$), and a side chain which varies with each amino acid, referred to as the 'R' group. **Peptide bonds** form between the amino ($-\text{NH}_2$) and carboxyl ($-\text{COOH}$) groups of adjacent amino acids to make chains called peptides (dipeptide = 2 amino acids linked together, polypeptide = many amino acids). As in carbohydrates, these linkages are made by a condensation reaction involving the elimination of a molecule of water.



Primary structure

The amino acid sequence is the starting point or primary structure of proteins. The final shape of proteins depends upon additional bonds which form between the -NH, -C=O, and -R groups which 'stick out' of the sides of the polypeptide chain.

Secondary structure

The secondary structure of proteins is determined by hydrogen bonds between adjacent -NH and -C=O groups. Two very different formations are possible, the **alpha helix** and **beta sheet**. Both of these secondary structures may occur in the same protein

Tertiary structure

The tertiary (third level) structure of proteins is determined by bonds which form between R groups. There are three possible types depending upon the chemical properties of the different -R groups. -R groups may be acid, basic, polar or non polar.

Quaternary structure

The quaternary (fourth level) structure of proteins arises from the combination of separate polypeptide chains to form a larger complex, e.g. haemoglobin, which is composed of four polypeptide chains with iron containing haem units.

The relationship of tertiary structure to function

The types of bonding which hold protein molecules in shape determine the shape and properties of the protein.

Hydrogen bonds are formed between polar -R groups (in addition to the H-bonds already described)

Ionic bonds are formed between the negatively charged acidic -R groups and positively charged basic -R groups. The forces are like those which exist between the Cl^- and Na^+ ions in salt.

Hydrophobic bonds occur between the -R groups of non polar amino acids e.g. valine (Val) and leucine (Leu).

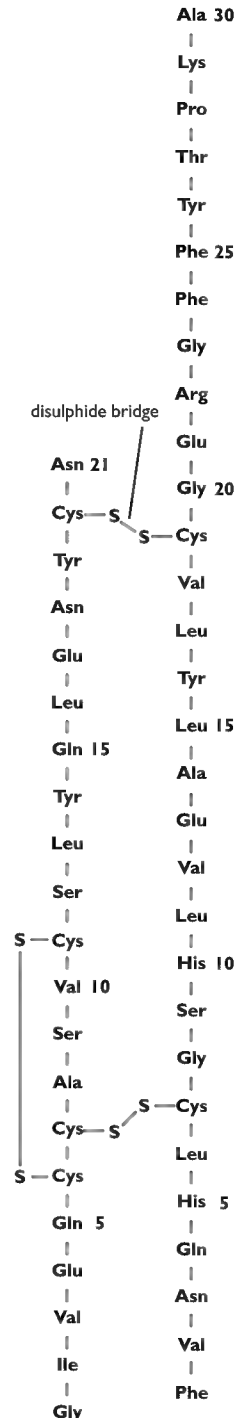
Disulphide bridges are much stronger covalent bonds formed between the sulphur atoms of molecules of the amino acid cysteine (Cys).

Proteins may be modified further by being linked to mineral ions, iron in the case of haemoglobin, or joined to carbohydrates and lipids to form **glycoproteins** and **lipoproteins**. This 'finishing off' process happens within the endoplasmic reticulum of cells.

Globular proteins

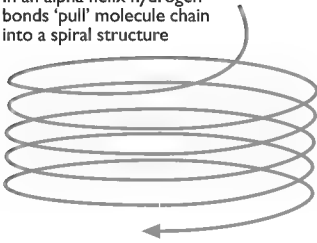
Globular proteins have a pronounced tertiary structure with many folds and twists, making them very susceptible to temperature and pH changes. Non polar groups tend to be repelled by water (hydrophobic) and tuck inside the molecule whilst the polar groups are attracted to water (hydrophilic) and they face outwards forming hydrogen bonds with water molecules. Globular proteins therefore tend to be soluble. Globular proteins are involved in metabolic activities e.g. enzymes, hormones, antibodies, and haemoglobin. **Haemoglobin** has a complex structure made up from four separate polypeptide chains (globins) joined to four iron (haem) ions. The iron (haem) groups in haemoglobin give the molecule its red colour and it is to these four sites that oxygen binds.

Primary structure

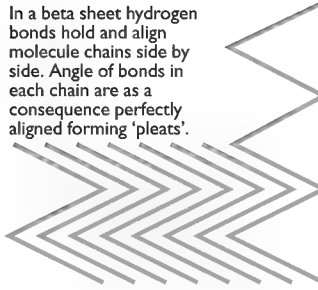


Secondary structure of proteins

In an alpha helix hydrogen bonds 'pull' molecule chain into a spiral structure

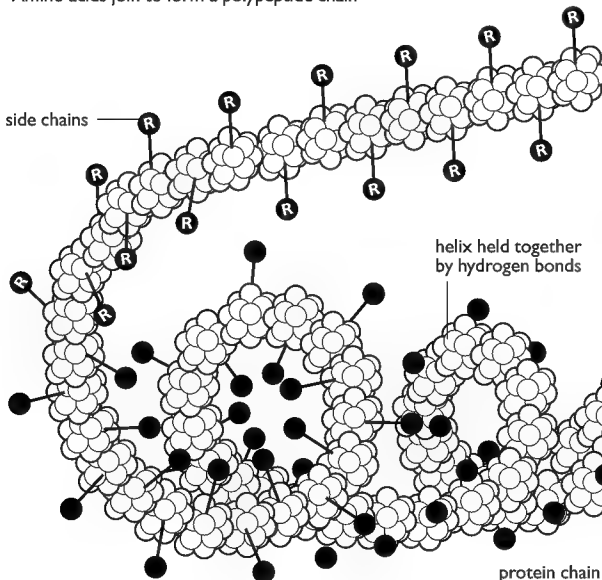


In a beta sheet hydrogen bonds hold and align molecule chains side by side. Angle of bonds in each chain are as a consequence perfectly aligned forming 'pleats'.



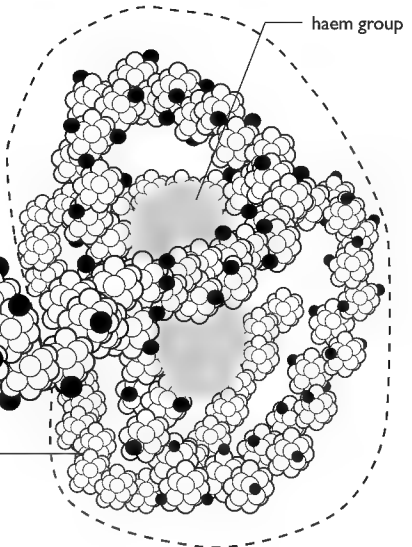
Primary structure

Amino acids join to form a polypeptide chain



Tertiary structure

Helix folds to form compact globular unit around Haem group



Secondary structure

Polypeptide chain folds in upon itself to form a helix

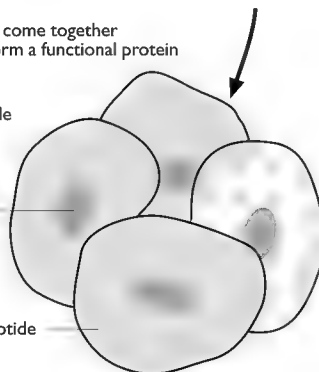
Quaternary structure

Several polypeptide chain units come together held by disulphide bridges to form a functional protein

Haemoglobin molecule has four subunits

haem group

globular polypeptide subunit

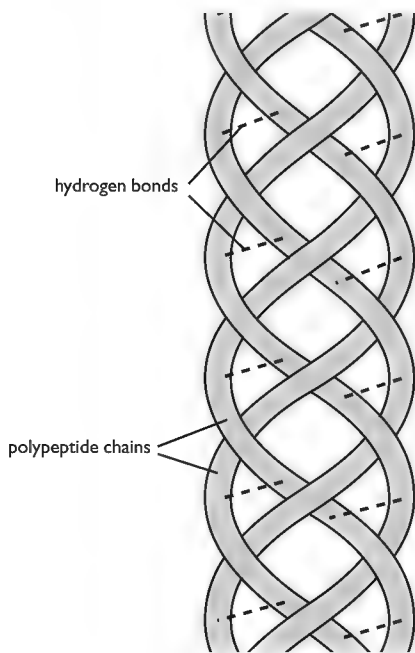


Fibrous proteins

In fibrous proteins the peptide chains are extended and inter-twined to form long thread like molecules. They do not dissolve in water and are relatively unaffected by changes in pH or temperature (they are not easily denatured). Fibrous proteins are mainly structural in function, giving support to tissues e.g. collagen in skin and bone, and keratin in skin and hair.

Test for Protein - the biuret test

2cm³ of dilute potassium hydroxide is added to 2 cm³ of the tissue extract in a test tube followed by a few drops of dilute copper sulphate solution. The presence of protein is indicated by a change in colour from light blue to purple (these colours may be interfered with by the colour of a food extract).



LIPIDS

Lipids are a family of molecules which include fats, oils, waxes, phospholipids and steroids. Although they vary in chemical structure they share two important characteristics: they are energy rich substances, and they are insoluble in water.

Structure and function of a triglyceride

Fats and oils are built on the same basic chemical plan which consists of a molecule of **glycerol** joined to three **fatty acid** chains. They are **triglycerides** ('mono' and 'diglycerides' are structures with one and two fatty acid chains respectively). The fatty acids are linked by **ester bonds** to glycerol in a condensation reaction similar to that seen in the formation of polysaccharides and polypeptides.

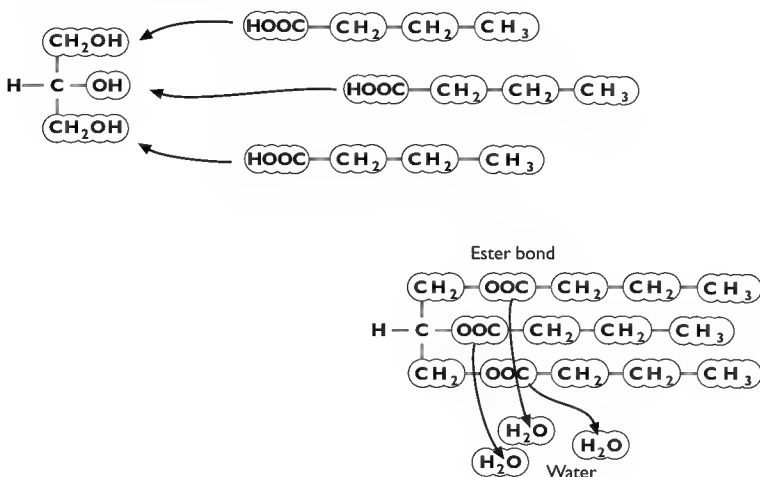
◆ CHECKPOINT SUMMARY

- ◆ All amino acids share a common structure consisting of a hydrogen atom, an amino group (NH₂), a carboxyl acid group (COOH), and a 'R' group, all attached to a carbon atom.
- ◆ The 'R' group can range from a single hydrogen atom to a long complex chain. Thus the simplest amino acid has the formula of CH₂NH₂COOH.
- ◆ Amino acids combine in a condensation reactions which form a peptide bond
- ◆ There are about 20 different types of amino acids found in proteins of living organisms
- ◆ Proteins are polymers of up to 2000 amino acids joined by peptide bonds (polypeptides)
- ◆ The combination of the 20 different types of amino acids gives rise to as many as 100 000 different types of proteins in living organisms.
- ◆ The sequence of amino acids in the polypeptide chain determines the primary structure of proteins
- ◆ Hydrogen bonds between adjacent NH₂ and COOH groups result in the secondary structure of either alpha helix or beta sheet. Both of these structures may occur in the same protein
- ◆ Bonds that form between the 'R' groups determine the tertiary structure
- ◆ Combination or association of two or more polypeptide chains determines the quaternary structure of a protein
- ◆ Haemoglobin has a quaternary structure involving four polypeptide chains and four iron containing haem groups.

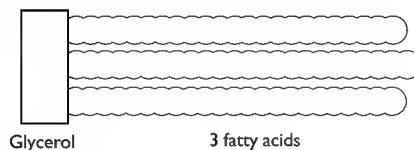
Fatty acids are hydrocarbon chains with a carboxylic (-COOH) group at one end. It is the -COOH group which forms the ester bond with glycerol. Hydrocarbons are non polar and, like fuel oils which have a similar structure, they contain a lot of energy locked up in their C-C and C-H bonds. Fats are mostly of animal origin and are solid at room temperature (RT); oils occur mostly in plants and are liquid at RT. The difference in melting point is due to the different nature of the hydrocarbon chains. In fats, each of the possible bonding sites between carbon and hydrogen is fully occupied (they are **saturated**), but in oils, one or more double bonds occur between the carbon atoms in the chain (they are **unsaturated**). Wherever a double bond occurs, the fatty acid chain acquires a kink, so the fatty acid chains of oils occupy more space than those of fats, giving them greater mobility and a lower melting point so that they are liquid at RT. The terms 'mono', 'di', and 'polyunsaturated' refer to the number of double bonds in the fatty acids.

When more food energy is taken in by the body than can be used, it may be converted to fat which is stored in large **adipose** cells under the skin and around internal organs. Adipose tissue not only acts as a long term food store; it also insulates the body against rapid changes in temperature, gives mechanical protection to internal organs and, acts as a buoyancy aid in aquatic animals.

Molecular model of development of a triglyceride



Model of a triglyceride

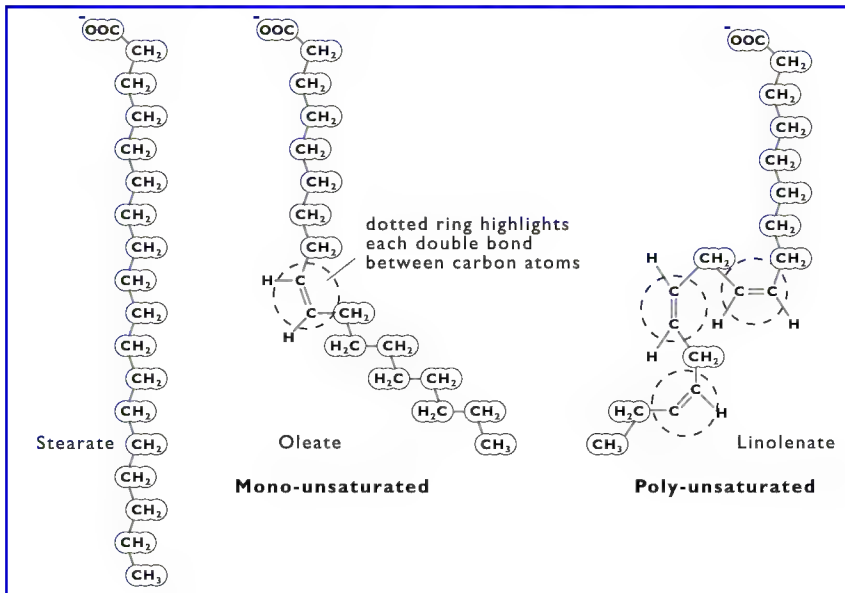


Waxes differ from fats and oils in that they have only one fatty acid chain and it is not joined to glycerol, but to a longer chain alcohol. They are produced in many animals and plants as a waterproof layer on external surfaces acting to reduce water loss (e.g cuticle of plant leaves, insect exoskeleton).

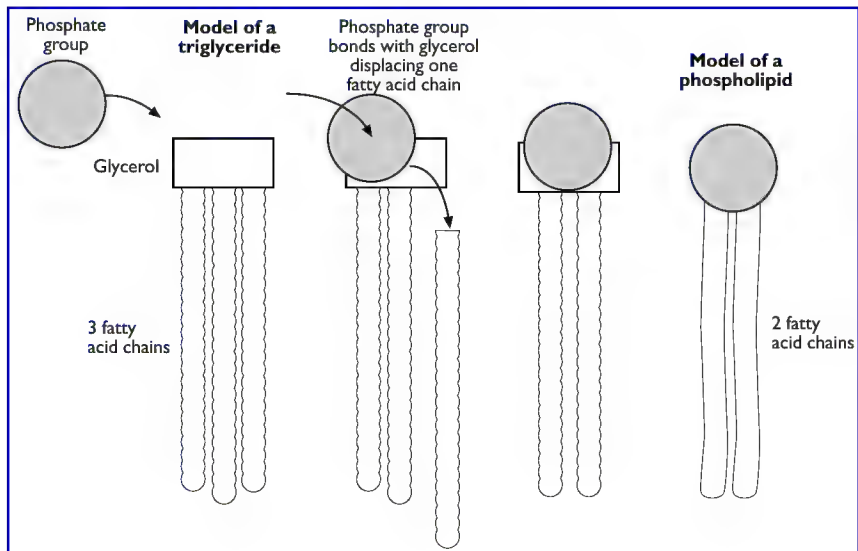
Structure and function of phospholipids

Phospholipids are essential to cells because they form the structural basis of all membrane systems. They have two fatty acid chains (which may be saturated or unsaturated) joined to a molecule of glycerol, the third position being occupied by a phosphate group (phosphate plus choline or ethanolamine).

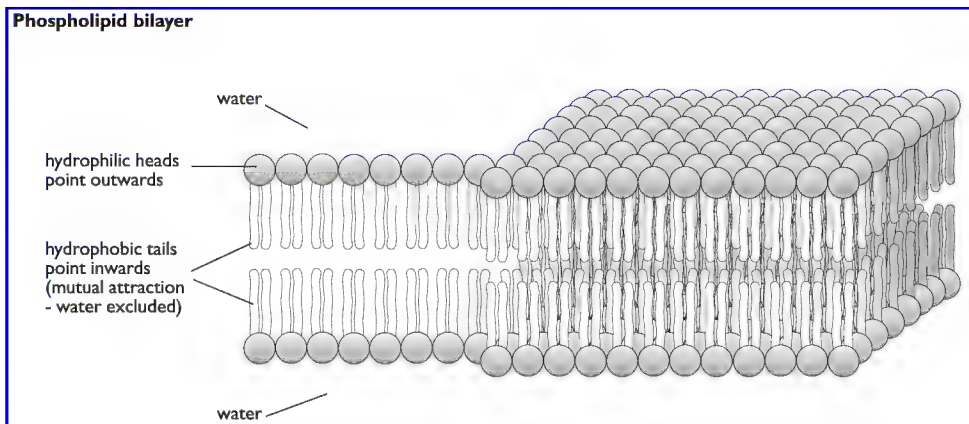
The phosphate group is strongly polar and readily dissolves in water, this is the hydrophilic (water loving) 'head' region of phospholipids. The non polar fatty acids are repelled by water and project in the opposite direction, and these are the hydrophobic (water hating) 'tails'. As a result, in the presence of water, phospholipid molecules form spontaneously into a characteristic **bilayer**. It is this bilayer which forms the molecular framework of all cell membranes.



Phospholipids



Phospholipid bilayer



Cholesterol is another important lipid component of cell membranes although it is very different in chemical structure to the lipids so far described. It has a ring structure and belongs to a group called **steroids** which include the sex hormones testosterone and oestrogen. Cholesterol molecules are strongly hydrophobic and they take refuge between the tails of phospholipids in membranes. Their presence in the bilayer has two effects. It slows down the lateral movement of individual phospholipid molecules, making the bilayer more stable, and it prevents the movement of certain substances through the bilayer, ensuring that membrane transport occurs through the correct (protein) channels.

Test for Lipids- the Ethanol Emulsion test

The tissue sample is ground in ethanol to dissolve any fats and oils. 2 cm³ of the filtered extract is then added to 2 cm³ of water in a test tube at room temperature. The presence of lipids is indicated by the formation of a milky white emulsion.

CHROMATOGRAPHY

The technique of chromatography to separate and identify molecules

The separation of different substances in a mixed solution can be achieved by chromatography or electrophoresis. These techniques exploit the fact that different molecules and ions tend to move at different rates through a paper or gel medium.

Paper Chromatography and R_f values

In this method, a concentrated sample of the mixture under study is formed by repeatedly placing small drops of it onto the same spot near one end of a piece of filter paper, letting the drop dry each time. The end of the paper is then dipped in a solvent solution such as ethanol and water. As the solvent front moves up the paper, so substances dissolved in it are carried along. The rate of movement for each substance depends upon its solubility in the solvent relative to water, so different substances move different distances depending upon how easily they enter the water phase associated with the cellulose fibres of the paper. The paper is removed from the solvent before it reaches the top, then the solvent front is marked and the paper, now called a **chromatogram**, is allowed to dry.

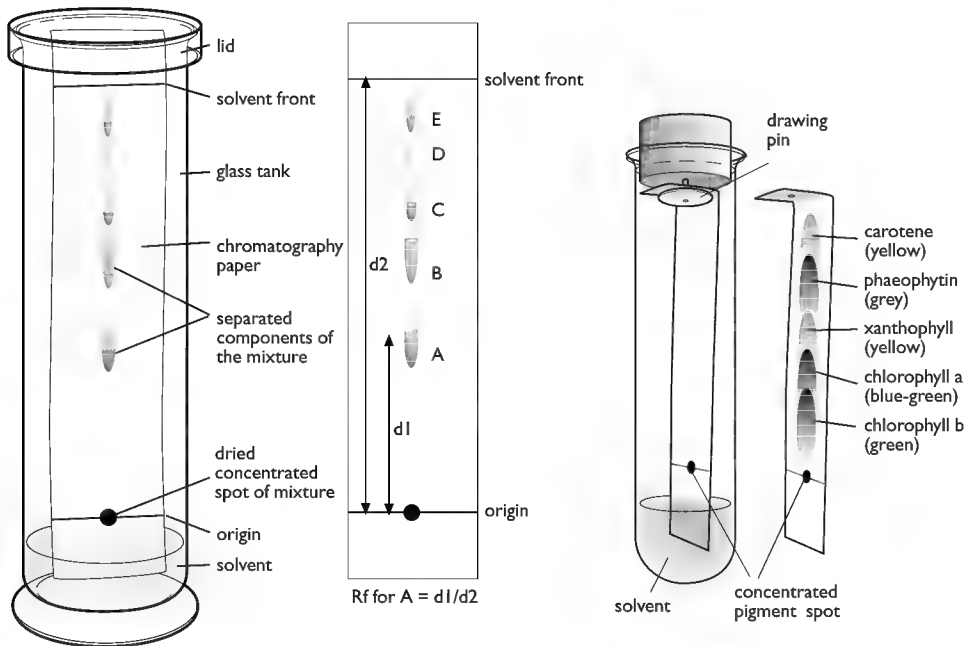
By comparing the distance travelled by the solvent with the distance travelled by each component of the mixture, a comparative value, called the R_f (relative front) value can be obtained.

$$R_f = \frac{\text{distance moved by the a substance from its origin (d1)}}{\text{distance moved by the solvent front (d2)}}$$

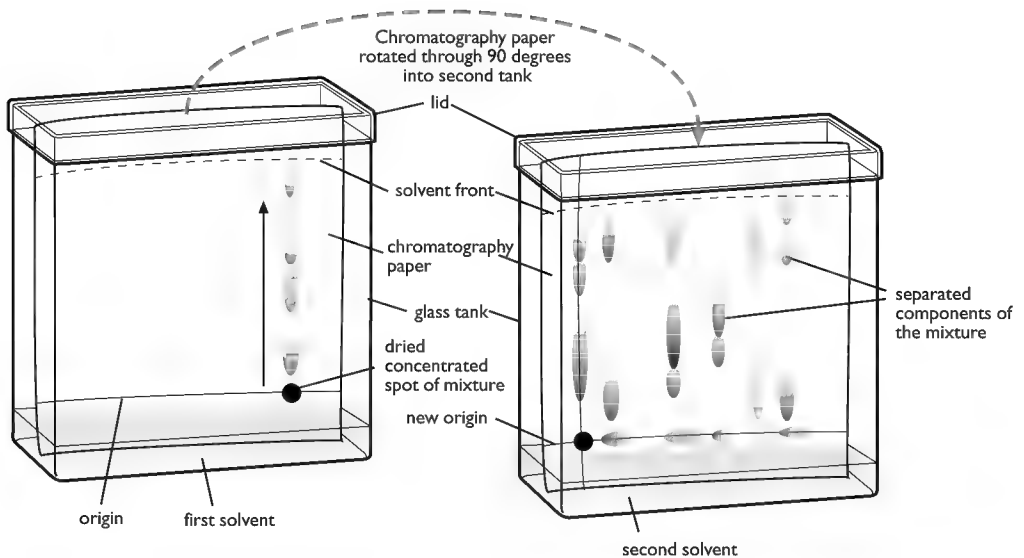
Two way chromatography

The separation may be enhanced by performing a second run of solvent, this time at right angles to the first, producing a **two way chromatogram**.

Separation of photosynthetic pigments



Two way chromatography



Thin Layer (gel) Chromatography

A slightly more sophisticated and faster version of paper chromatography, this technique makes use of a sheet of glass coated with an absorbent gel (e.g silica or starch). The glass plate is dipped into a solvent just like the paper in the method described above.

Examples of the use of chromatography in biology include the separation of photosynthetic pigments from plant leaf extracts, and the separation of amino acids from a protein sample. A concentrated sample of photosynthetic pigments can be obtained by grinding up leaves in a solvent such as acetone or ethanol. The different component molecules separate out in the chromatogram as clearly visible spots of different colours.

Proteins selected for this treatment must first be hydrolysed to break up the polypeptide chain into its constituent amino acids. This is done by soaking in hydrochloric acid for 24 hours. As amino acids have no colour of their own, they must be stained on the final chromatogram with a dye called **ninhydrin**. The colour intensity of each stained blob on the chromatogram can be measured using a colorimeter to reveal the proportion of each amino acid in the protein.

◆ CHECKPOINT SUMMARY

- ◆ Paper Chromatography is used to separate small traces of materials from a mixture
- ◆ A concentrated spot of the mixture is placed near one end of a piece of chromatography (or filter) paper. The end of the paper is then dipped in a solvent solution such as ethanol and water
- ◆ As the solvent front moves up the paper, so substances dissolved in it are carried along
- ◆ The rate of movement for each substance depends upon its solubility in the solvent relative to water, so different substances move different distances depending upon how easily they enter the water phase associated with the cellulose fibres of the paper
- ◆ The paper is removed from the solvent before it reaches the top, then the solvent front is marked and the paper, now called a chromatogram, is allowed to dry
- ◆ By comparing the distance travelled by the solvent with the distance travelled by each component of the mixture, a comparative value, called the R_f (relative front) value can be obtained
- ◆ The separation may be enhanced by performing a second run of solvent, this time at right angles to the first, producing a two way chromatogram
- ◆ A slightly more sophisticated and faster version of chromatography, this technique makes use of a sheet of glass coated with an absorbent gel (e.g silica or starch). The glass plate is dipped into a solvent just like the paper in the method described above
- ◆ Examples of the use of chromatography in biology include the separation of photosynthetic pigments from plant leaf extracts.

1.5

Enzymes as proteins which control biochemical reactions in cells

Contents

Enzyme action

- ▼ The protein nature of enzymes
- ▼ Enzymes as catalysts lowering activation energy through the formation of enzyme-substrate complexes.
- ▼ The lock and key and induced fit models of enzyme action.

Factors which affect enzyme activity

- ▼ The effects of temperature, pH, substrate and enzyme concentration, competitive and non-competitive inhibitors.

ENZYME ACTION

The protein nature of enzymes

Most students of science can name an enzyme. Usually it is a digestive enzyme like amylase or pepsin, but enzymes are not just concerned with breakdown reactions like digestion, they can also help build up molecules. In fact, every chemical reaction in every living cell is made possible by an enzyme. Several thousand enzymes have been isolated and studied.

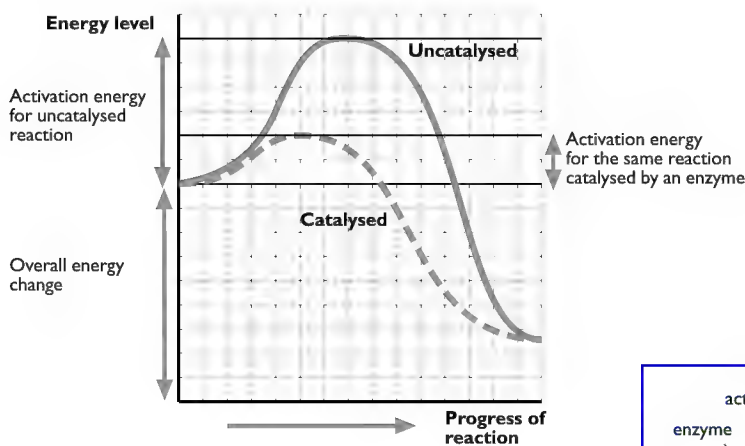
Enzymes are protein molecules. Compared to many other molecules they are relatively large (macromolecules) of the globular type described in 10.4 with a complex tertiary structure. What gives each enzyme its particular qualities and mode of action is the way in which its polypeptide chains are folded and twisted into specific shapes. As you will see in the following account, enzyme action depends upon shape recognition between enzymes and reacting molecules called **substrates**.

Enzymes as catalysts lowering activation energy through the formation of enzyme-substrate complexes

Enzymes are **biological catalysts**. A catalyst is a substance which speeds up a reaction without being changed itself in the process. As they are not used up or changed, enzymes can be used repeatedly, therefore they are effective in relatively small amounts.

Usually enzyme molecules are much larger than the reacting molecules (substrates). In an enzyme-catalysed reaction, the substrate(s) become attached to a particular region on the enzyme called the **active site** which has a specific shape and structure matching that of the substrate(s), forming the **enzyme - substrate complex**. This association is temporary and lasts only long enough to allow the substrate molecules to interact to form the products. Once the products are formed, they are released from the active site and the enzyme is free to repeat the process.

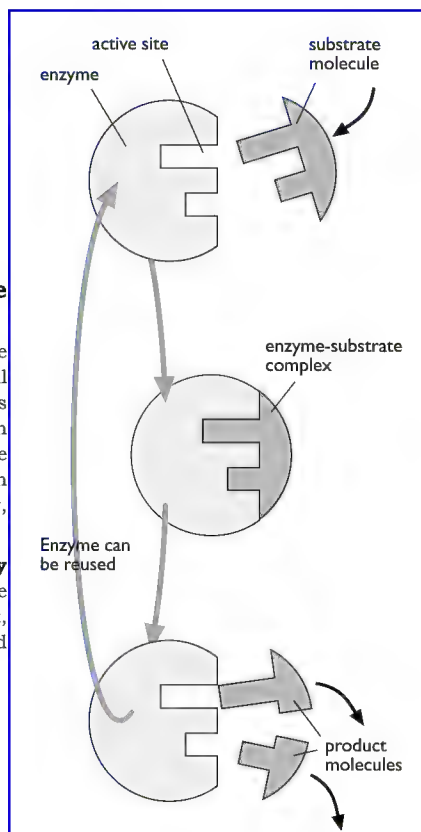
The formation of the enzyme-substrate complex lowers the **activation energy**, which is the energy needed to start a reaction. In the laboratory, it is common for reacting substances (substrates) to be heated in order to make the reaction occur more quickly. The heat energy causes the molecules to move about more rapidly (i.e. it gives them more kinetic energy) so that they collide more frequently, forming the product. In other words heat is used to reduce the activation energy. Living cells operate at a constant relatively low temperature so therefore they rely on enzyme catalysts to overcome the activation energy.



The lock and key and induced fit models of enzyme action

The active site matches the substrate, so different shaped substrate molecules require different shaped active sites. Most biological reactions therefore involve different, **specific** enzymes. This is possible because proteins which make up enzymes can be made in an infinite variety of shapes as a result of the nature and sequence of the amino acids and their R group (primary structure) which in turn leads to the development of a complex 3D structure (secondary, tertiary and quaternary structures).

This model of enzyme action is referred to as the **lock and key model**, but it is known that the shape of the active site may change as the substrate makes contact with it, adjusting to make a close fit, rather like a soft glove around a hand, a mode of action called **induced fit**.



FACTORS which affect ENZYME ACTIVITY

Temperature

Temperature affects the rate of all chemical reactions. As the temperature increases, so does the kinetic energy of the reacting molecules. More enzyme-substrate complexes are formed as the reactants collide more frequently, and the reaction rate rises exponentially, doubling for every 10°C rise in temperature. However, enzymes, like all proteins, suffer irreversible alterations in molecular shape above certain temperatures as a result of the breaking of chemical bonds within the molecule, so that in enzyme-catalysed reactions, there is an **optimum** temperature above which the reaction rate drops sharply. For an enzyme, any change in the shape of the active site means a loss of function, the enzyme is said to be **denatured**. For most enzymes, the optimum temperature is around 40°C, but there are extreme exceptions. For example, some bacteria living in hot springs have enzymes which function at temperatures above 85°C, whilst the ice fish of the Antarctic possess enzymes which operate at -2°C.

pH

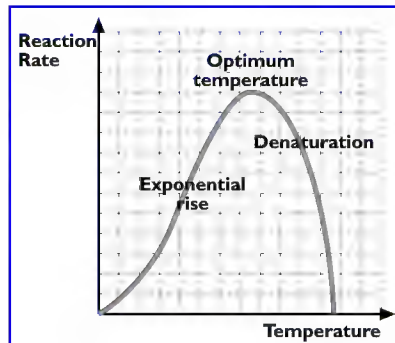
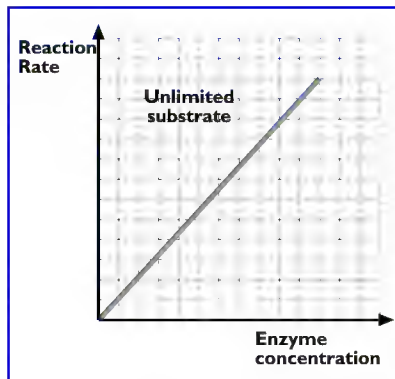
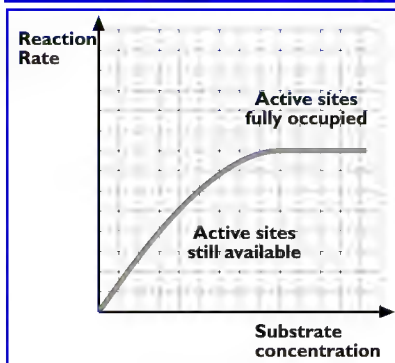
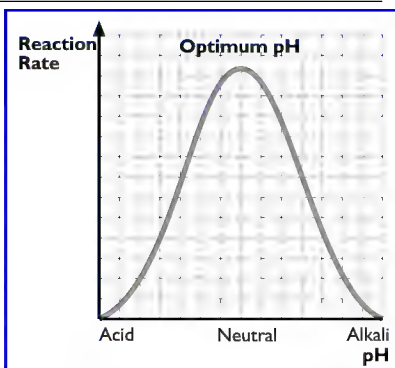
This is a measure of hydrogen ion (H^+) concentration, which is a measure of acidity and alkalinity. A pH of 7 is neutral, less than 7 is acid, and more than 7 is alkaline. Small changes in pH can affect the association process between enzyme and substrate which happens at the active site. The attraction between substrate and enzyme is often the result of small electric charges at the active site, and these are disrupted by changes in H^+ ion concentration. Extremes of pH, i.e. very acid or alkaline conditions, cause permanent denaturation. Enzymes have an optimum pH for efficient functioning. Usually this is around pH7, the normal pH of cells. Notable exceptions are the enzyme pepsin, which is found in the stomach and works best in highly acidic conditions in the range pH 1-2, and the enzyme trypsin which is found in the duodenum and has an optimum pH of 9.

Substrate concentration

Substrate concentration exerts a direct effect on the rate of the reaction. At low substrate concentrations, not all the active sites will be occupied, so the rate of the reaction depends upon the concentration of substrate alone. As the concentration of substrate increases, so all of the available active sites will become filled. The upper limit is determined by the amount of enzyme molecules available.

Enzyme Concentration

Enzyme concentration has a similar effect. As long as there is a plentiful supply of substrate, any increase in the number of enzyme molecules will result in a proportional increase in the number of enzyme-substrate complexes and therefore an increase in the rate of reaction.



Enzyme Inhibitors

Enzyme inhibitors are substances which interfere with the active site of the enzyme. They do this either directly by blocking it, or indirectly by attaching to another part of the enzyme and altering its molecular shape so that the enzyme-substrate complex cannot be formed.

Competitive inhibitors

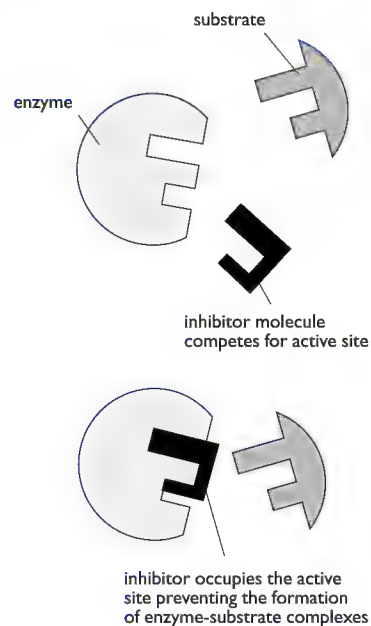
These are substances which have a molecular structure resembling that of the substrate. They attach themselves to the active sites forming inhibitor-enzyme complexes and cause the reaction to slow down or stop altogether. The effect of competitive inhibitors depends upon the relative concentrations of the substrate and inhibitor, and also the relative stability of the enzyme-substrate and inhibitor-enzyme complexes. This type of inhibition is seen in the action of a group of antibiotics called sulphonamides, which compete for the active site of a key bacterial enzyme involved in synthesising an essential growth factor, not found in the cells of the host.

Non-competitive inhibitors

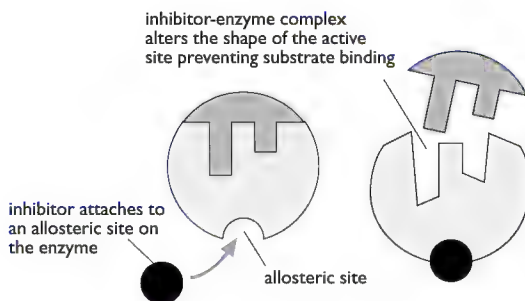
These are substances which bind onto the enzyme at a place other than the active site called an **allosteric site**, causing the enzyme to change shape sufficiently to stop enzyme-substrate complexes being formed. Non-competitive inhibitors are not similar to the substrate molecules and are not affected by the substrate concentration. The ions of some heavy metals such as arsenic and cyanide may bind onto allosteric sites and act as poisons, stopping important enzyme controlled reactions. Some enzyme molecules possess an allosteric site which must be occupied by an **activator** substance to give the enzyme its correct working shape. Without the activator, the enzyme will not work.

Inhibitors and activators are important in regulating the activity of enzymes.

Competitive inhibitor



Non-competitive inhibitor



◆ CHECKPOINT SUMMARY

- ◆ All enzymes are protein in nature, and fall into the group of 'globular' proteins with a complex 3D structure
- ◆ Enzymes are catalysts which speed up the rate of reaction of much larger amounts of reactants without themselves being used up in the process
- ◆ Unique amongst catalysts they are denatured by extreme conditions of pH and temperature etc.
- ◆ Their activity depends upon their complex 3D shape which creates an active site which combines with the substrate to form an enzyme/substrate complex which lowers the activation energy of reactants
- ◆ Their complex 3D shape also accounts for enzyme specificity as the active site has a shape complementary to the shape of the substrate
- ◆ Disruption of the 3D shape accounts for denaturation.

1.6

Tissues and organs

Contents

Epithelial tissue

- ▼ Epithelial tissue as a lining tissue
- ▼ Epithelial tissue as exemplified by the alveolar epithelium which acts as a surface for gas exchange

Blood

- ▼ Blood cell types
- ▼ The structure of red blood cells in relation to their transport function
- ▼ The relationship between size and surface area to volume ratio

Blood vessels

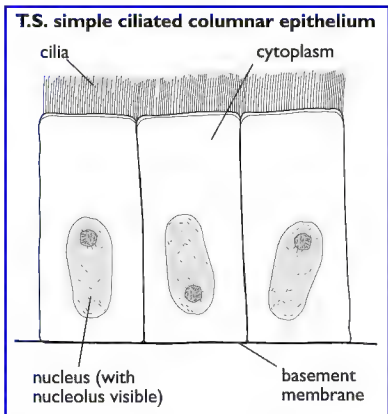
- ▼ Blood vessels as organs
- ▼ The structure of arteries, arterioles and veins in relation to their function.

EPITHELIAL TISSUE

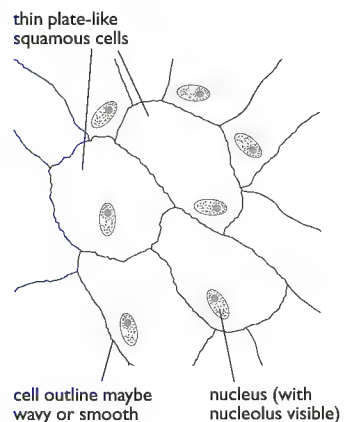
A **tissue** is a group of similar cells, specialised to perform the same function. Epithelial tissues cover both external and internal surfaces, and typically form sheets of large surface area but of thin cross-section. The cells are held together by an intercellular cement, and by tight junctions between adjacent membranes. At their base the cells are fixed to a basement membrane. They have one free surface which is often highly specialised with, for example, microvilli or cilia.

Epithelia may have a protective function, as in the skin, protecting underlying regions from the entry of pathogens and from the damaging effects of abrasion, desiccation and ultraviolet light, in these cases they are multilayered (compound). In the bronchial tubes, the epithelial lining is **ciliated** to move trapped foreign particles and prevent their entry into the lung, whilst in the alveoli, the epithelium is adapted to facilitate gas exchange.

Gas exchange in the human lungs occurs over a surface formed by around 700 million tiny air sacs (**alveoli**), each constructed out of epithelial cells. This gives an estimated total surface area of 70-90m². The **bronchiole**, leading to the alveolar air sac, in common with all the air passages, is lined by **ciliated** and **glandular epithelia**. Foreign particles are trapped by mucus secreted by the glandular cells and moved up the respiratory tubes towards the mouth by the beating action of the cilia. The surfaces of the alveoli are further protected by phagocytic cells called macrophages which move over them in amoeboid fashion, engulfing bacteria and other particles. Each air sac is constructed from **squamous** (flattened) epithelial cells allowing the shortest possible diffusion distance between the air space and the blood capillaries.



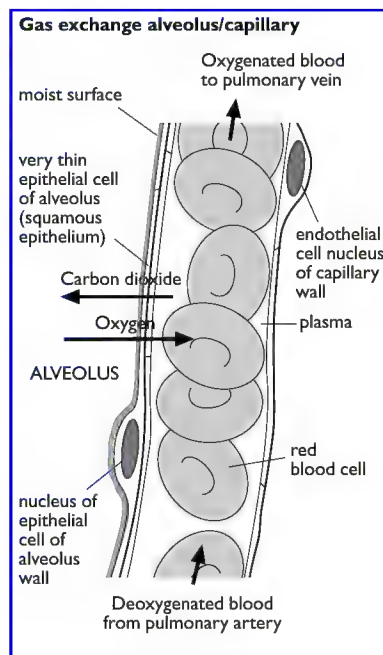
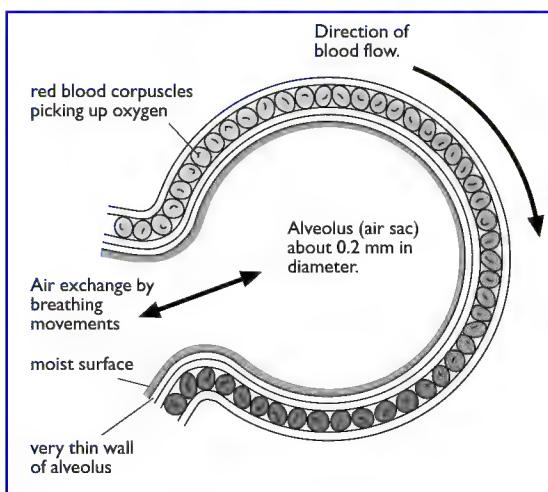
Surface view of simple squamous epithelium



In section squamous cells look like slender rods thickened where the nucleus is located

Each alveolus is surrounded by a network of capillaries which together form the **alveolar-capillary complex**. As this name suggests, the endothelium lining of the capillaries is attached to the thin squamous epithelium of the alveolus via a shared basal membrane. The air inside the alveolus and the blood in the capillaries is thus separated by a distance of just the thickness of two layers of very thin cells. This is one feature that helps rapid diffusion of oxygen from alveolus to blood and carbon dioxide from blood to alveolus. Other features include the large surface area already mentioned, and the steep diffusion gradients which are maintained between the blood and the alveolus due to the constant movement of the blood and the constant ventilation of the lungs.

The presence of a phospholipid material called **pulmonary surfactant** ensures that the tiny air sacs do not stick together like wet panes of glass when they collapse, but slide over each other maintaining a clear airway. Phospholipids act like a detergent, reducing the surface tension of fluids at the alveolar surface.



BLOOD

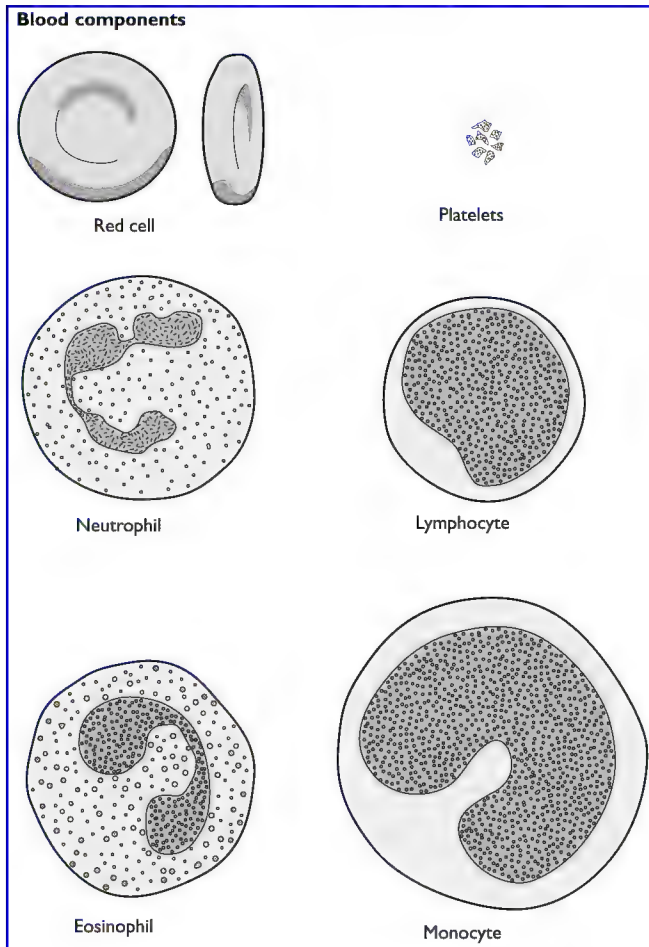
Blood Cell types

Blood is a specialised tissue containing a number of different cell types

The structure of red blood cells in relation to their transport function

Of the cellular components, red blood cells (erythrocytes) are by far the most numerous. Red blood cells lose their nucleus and most of the cytoplasmic organelles in their process of maturation, and become 'corpuscles' with a flexible membrane, filled with the oxygen carrying pigment haemoglobin. The loss of the nucleus accounts for their biconcave shape which increases their **surface area to volume ratio**. This ratio is of the utmost importance for any cell or group of cells exchanging materials with their surroundings. In general, as the volume of a cell increases so its surface area to volume ratio decreases. This principle is illustrated by the fact that the surface area to volume ratio halves as the sides of a cube are doubled from 1 to 2 cm.

The total surface area of the red blood cells in man is about 3500 m² and this helps the exchange of gases between the red cells and the tissues. The biconcave shape also gives them some resilience in absorbing water without bursting should the plasma become temporarily diluted for any reason, and the flexible membrane enables them to 'squeeze' through capillaries.



BLOOD VESSELS

Blood vessels as organs

An organ may be defined as a structure consisting of several tissues which together perform a complex function (examples include the stomach, brain and lung). Blood vessels are organs whose function is the circulation of blood throughout the body. The blood circulates around a continuous system of blood vessels, from the heart in the arteries and arterioles, into the capillary beds supplying the tissues, and back to the heart in the venules and veins.

◆ CHECKPOINT SUMMARY

- ◆ In multicellular organisms cells are organised into tissues. A tissue being an association of similar cells specialised and coordinated to a particular function. Some functions being more specialised than others
- ◆ Examples of tissues in mammals include squamous epithelium covering surfaces e.g. in the kidney tubule and the alveoli of the lungs, ciliated epithelium lining the trachea and bronchi, cerebro-spinal canal, and oviduct
- ◆ Some tissues consist of only one type of cell. e.g. ciliated epithelium, whilst others consist of several variants of similar types of cells
- ◆ Cells in the same tissue develop from the same original cell type, even though the fully differentiated cells may appear very different
- ◆ Different tissues become 'organised' into organs specialised to particular functions, e.g. a lung is an organ and consists of squamous epithelium, ciliated epithelium, connective tissue, blood vessels etc.
- ◆ Blood consists of fluid plasma with red blood cells, white blood cells (or white cells) and blood platelets
- ◆ Plasma has 10% materials in suspension and solution
- ◆ Plasma proteins wide variety of functions and act to buffer changes in pH
- ◆ Materials carried in solution not strictly part of the plasma
- ◆ Red blood cells lose nucleus in development and become biconcave corpuscles of haemoglobin
- ◆ Large surface area to volume ratio individually due to biconcave shape, and huge total surface area due to small size (6-8 µm) and huge numbers
- ◆ Red blood cell volume 30-50% of total blood volume
- ◆ Males higher blood count/haemoglobin than females
- ◆ White cells of which there are several types form basis of immune system
- ◆ Platelets are fragments of white cells.

Arteries, arterioles and veins

The arteries close to the heart have a large cross sectional area, and thick elastic walls. Arteries are stretched by the cardiac output when the heart contracts, and they recoil as the arterial blood pressure drops when the heart relaxes between beats. The recoil of the elastic artery walls assists the circulation of the blood around the body, helps smooth the flow between contractions of the heart, and helps to maintain the blood pressure. This stretching and recoiling of the arteries is felt as the pulse in places where arteries come close to the surface of the body (the inside of the wrist, the side of the neck, and in the temple).

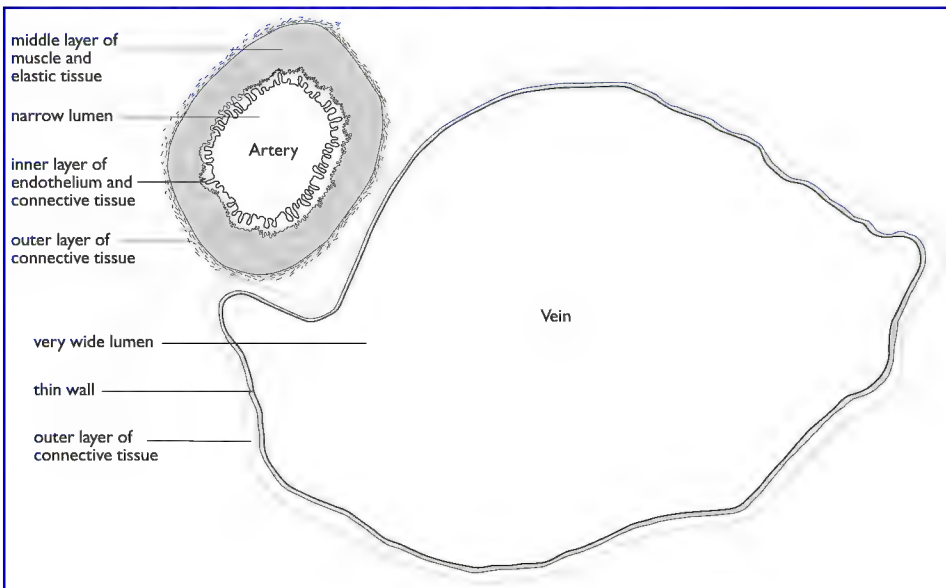
Further from the heart, the arteries branch into the smaller arterioles. The arterioles penetrate all tissues of the body and connect to beds of finely branching capillaries.

From the capillary beds **venules** join together to form **veins**, which return the blood to the heart. The individual veins have a larger cross section area than the comparable arteries, and as there are more veins than arteries, the veins also have a greater total cross sectional area. The walls of the veins are thinner and less elastic than those of the arteries, and in the veins of the legs and arms there are semi-lunar or pocket valves at intervals along their length. These valves oppose any back flow of blood under gravity, and ensure one-way flow of blood back to the heart.

The thin walled veins with their large cross section area present little resistance to the flow of the blood, and although after the blood has passed through the capillaries, the blood pressure in the veins is low, it still helps move the blood in the veins back to the heart. Because the blood in the veins is under low pressure, and their walls are thin, the veins are easily compressed by the smallest movements of the surrounding structures, particularly the skeletal muscles. As the muscles contract and relax during exercise they exert a massaging effect on the deep veins, especially for example in the legs, when running, swimming, or cycling. This massage effect of the 'muscle pump' is random and in no particular direction, but the pocket valves in these veins ensure that the flow is one-way, back to the heart.

◆ CHECKPOINT SUMMARY

- ◆ Elasticity of arteries important in smoothing flow of cardiac output. Expansions and contractions is felt as the pulse
- ◆ Finer branches and arterioles main site of generation of resistance to blood flow
- ◆ Capillary beds supply all tissues providing large surface areas for exchanges by diffusion and short diffusion distances
- ◆ Capillaries have large total cross sectional surface areas. Therefore resistance to flow, blood pressure and rate of flow all drop in capillaries
- ◆ Diameter of capillary same as red blood corpuscles thus forcing them to pass through capillaries in single file, bringing all close to tissues
- ◆ Plasma minus proteins is exuded through thin walls to bathe tissues directly.
- ◆ Thin walls and large lumen of veins offer little resistance to flow of blood back to heart, and allow ease of massage by surrounding muscles
- ◆ Pocket valves ensure one way flow back to heart
- ◆ Venous blood is under low pressure but the flow is fast enough to ensure return of blood to heart at same rate as blood flow in the arteries.



1.7

The blood system as a mass flow system

Contents

Circulation

- ▼ The general pattern of blood circulation in a mammal

Capillaries

- ▼ The structure of capillaries and their importance in metabolic exchange
- ▼ The formation of tissue fluid and its return to the circulatory system

Lung function

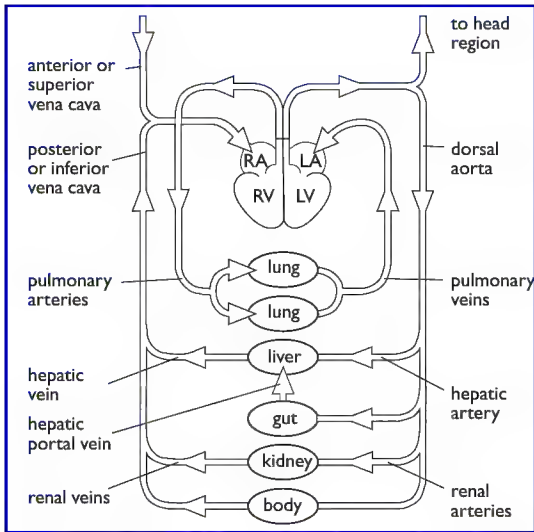
- ▼ The gross structure of the human gas-exchange system
- ▼ The exchange of respiratory gases in the lungs
- ▼ Fick's law

Ventilation

- ▼ Mechanism of ventilation and its nervous control
- ▼ The composition of inhaled and exhaled air
- ▼ The role of the medulla and the phrenic nerves in generating a basic breathing rhythm

CIRCULATION

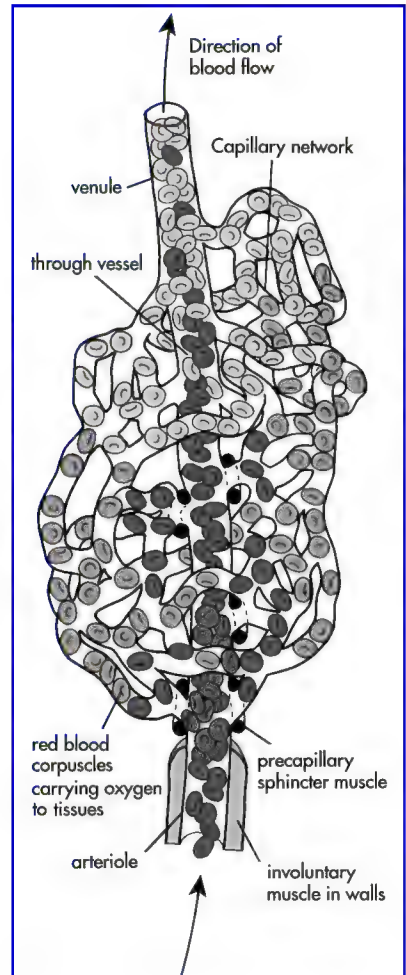
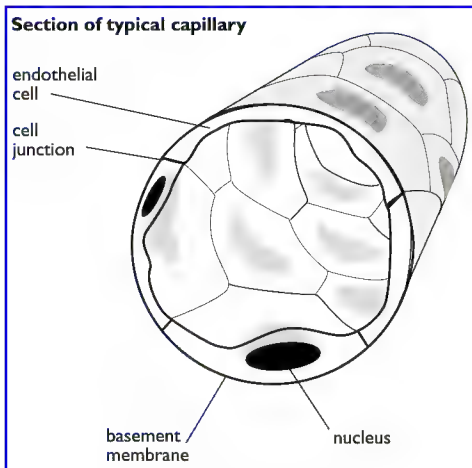
Transported materials are carried around the whole closed circulatory system, accumulating in high concentrations in those regions where they are absorbed or produced (**sources**) and reaching their lowest concentrations in areas where they are being used up or excreted (**sinks**). The overall flow of materials is from source to sink. This is described as **mass flow**. Arteries and arterioles deliver materials to the tissues, venules and veins carry blood back to the heart for oxygen uptake at the lungs and recirculation, but the important exchange of materials between blood and body tissues occurs in the capillary beds.



CAPILLARIES

The structure of capillaries and their importance in metabolic exchange

The capillaries form a fine network which penetrate all tissues. They have the smallest diameter of all blood vessels, about 7 micrometres (0.007 mm), which is the same as that of the red blood cells. For this reason the red cells are forced to move slowly through the capillaries in single file, ensuring a short diffusion distance between the corpuscles and the tissues. Capillary walls are just one cell thick, which further aids exchanges by diffusion between the blood and the tissues. The huge number of capillaries provide a very large surface area across which these exchanges occur. As more capillaries open up in working muscles, the surface area between the blood and tissues is increased, and the diffusion distance between the two is decreased, resulting in more rapid and efficient exchanges between them.



The formation of tissue fluid and its return to the circulatory system

Tissue fluid is formed when plasma, minus most of its proteins, is pushed out under pressure through the capillary walls. Depending on the degree of activity and health and fitness, most of the tissue fluid is reabsorbed back into the blood capillaries. Tissue fluid bathes each cell and is the medium for the exchange of materials between the individual cells and the blood system.

The tissue fluid which is not reabsorbed into the blood capillaries, especially any proteins, enters the lymphatic capillaries, which are found in all tissues, and becomes lymph. If the lymphatic capillaries did not drain the tissues, the blood protein would accumulate in the tissue fluid and, due to its osmotic effect, would hold increasing amounts of water in the tissues. Lymph has a higher protein content than the tissue fluid.

The blind-ended lymphatic capillaries lead into wider lymph vessels which are similar in structure to the veins, having thin walls with pocket valves. Contraction of skeletal muscles massages the vessels and the valves ensure unidirectional flow. This aids the draining of tissue fluids.

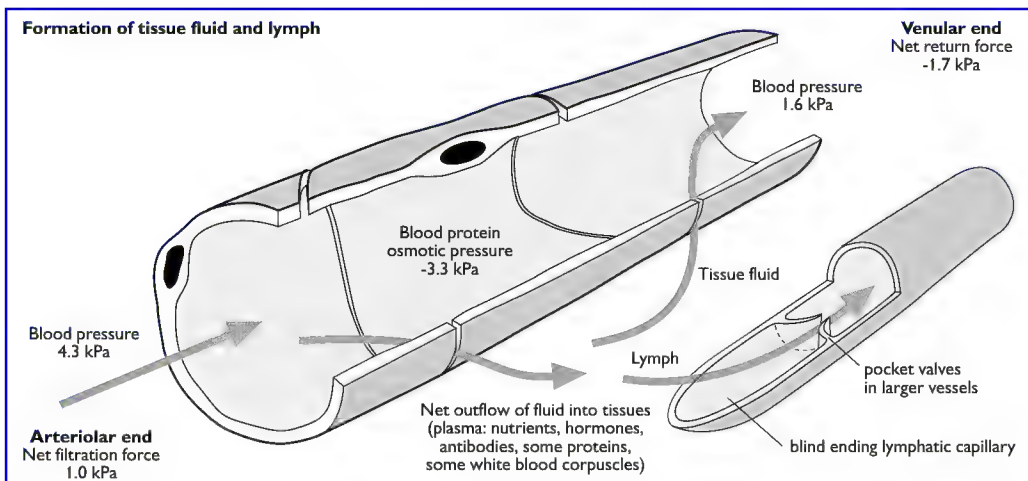
During exercise the blood pressure may increase, and more tissue fluid is exuded; the lymph vessels are massaged more vigorously, and therefore the tissue fluid is drained more quickly. All these factors increase the speed of supply of oxygen and nutrients to, and removal of waste products from, the tissues.

At intervals in the system are swellings called lymph nodes, which play an important role in the defence of the body against infection (ref 3HB 12.3).

The lymph is returned to the circulation in the anterior venae cavae.

◆ CHECKPOINT SUMMARY

- ◆ Closed double mammalian circulation more efficient than open single circulation
- ◆ Open circulation has blood returning to heart in large open blood spaces (haemocoel), slow flow and low pressure
- ◆ Single circulation blood only passes through heart once on each complete circulation of the body
- ◆ Means that blood passes through two sets of capillaries on each circulation, respiratory surface and body tissues
- ◆ Large resistance to overcome before blood returns to heart, resulting in slow flow and low pressure in veins which are typically large blood sinuses to lower resistance to flow
- ◆ Closed systems blood almost entirely restricted to vessels: arteries, arterioles, capillaries, venules, veins
- ◆ Tissue fluid exuded to bathe tissues directly
- ◆ Double circulation blood passes through heart twice on each circulation
- ◆ Pressure relatively low to lungs
- ◆ Pressure raised for systemic circulation to body giving high pressure and fast flow.



LUNG FUNCTION

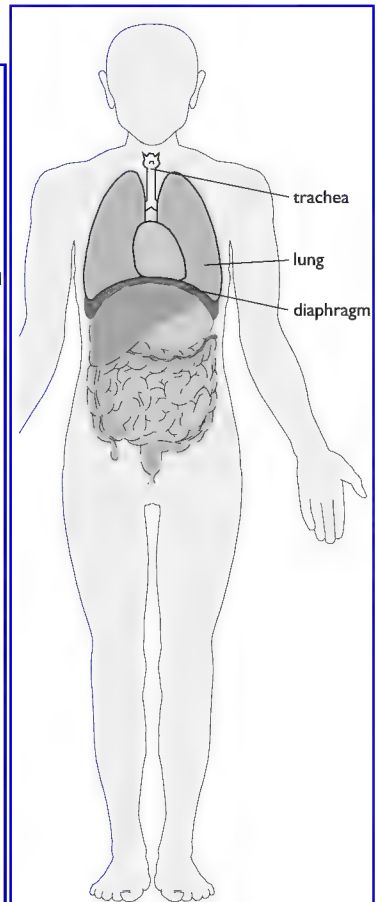
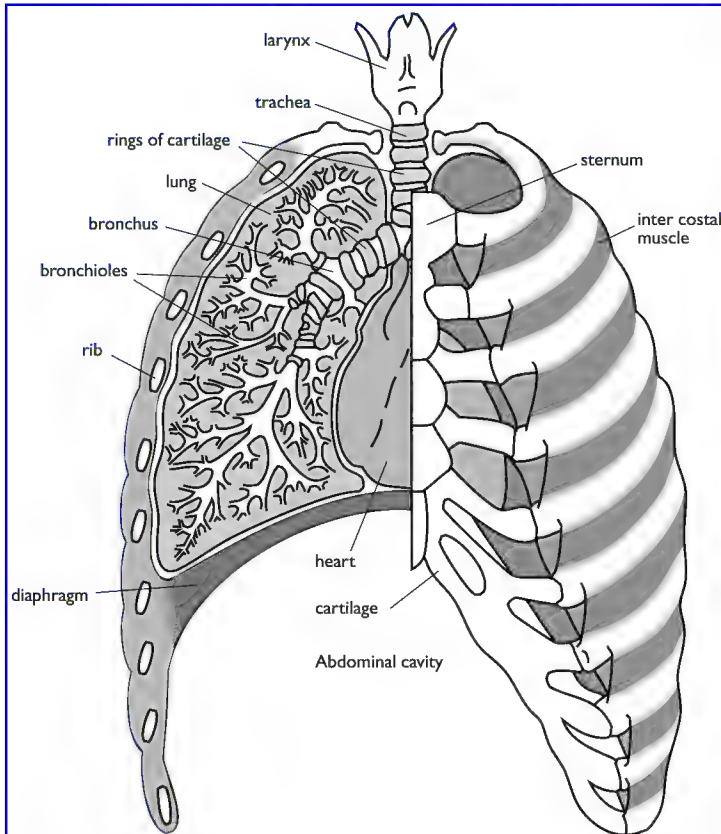
Structure of human gas exchange system

Together with the heart, the lungs fill all the available space in the air tight **thoracic (chest) cavity** bounded by the ribs and the muscular sheet known as the **diaphragm**. Air passes into a series of tubes which make up the respiratory system having first been warmed and moistened in the nasal cavity. The **trachea, bronchi and bronchioles** form a system of branching air tubes referred to as the 'bronchial tree'. The wall of the trachea and bronchi is composed of an **epithelial** layer, **connective tissue**, **smooth muscle** and **cartilage**, and a tough outer coat. Bronchioles differ in that they lack cartilage.

Cartilage supports the trachea and bronchi and prevents their collapse. In the trachea the cartilage forms C-shaped rings which are arranged so that the open ends of the C are at the back of the tube, facing towards the oesophagus and the spine. These support the tube and keep it open during inspiration (breathing in). Between the ends of the Cs at the posterior (back) of the trachea, smooth muscle completes the tube. The incomplete nature of these 'rings' of cartilage allows some flexibility as the oesophagus expands when food is moving down. In the bronchi the cartilage does not form rings but instead forms plates within the smooth muscle layer to strengthen the tubes.

◆ BLOOD PRESSURE

The continuous random movement of particles of a gas means that the particles will collide with each other and with the walls of any structure enclosing them within a space, thus exerting a pressure. The number of such collisions, and therefore the pressure, is proportional to the amount of substance present. In mixtures of gases, e.g. air, each substance exerts a partial pressure in that mixture proportional to the amount of that substance in the mixture.



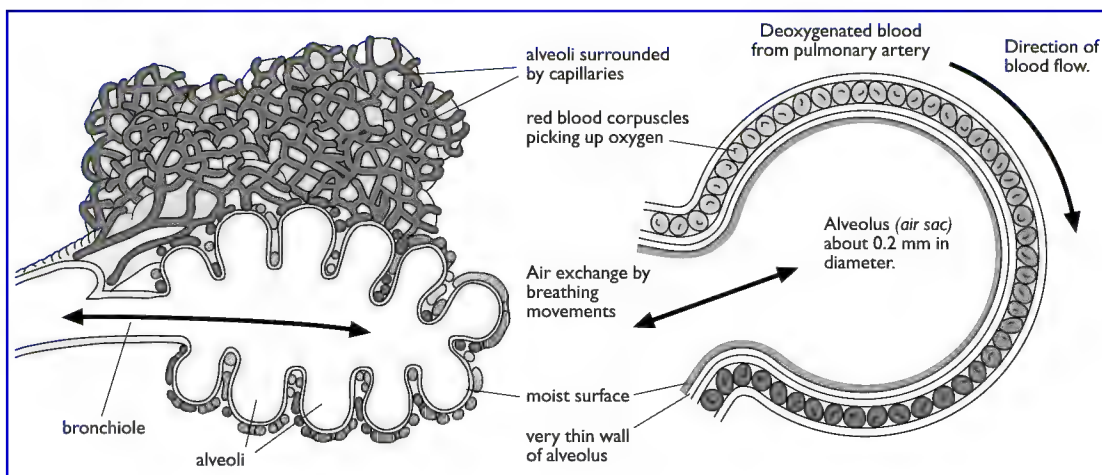
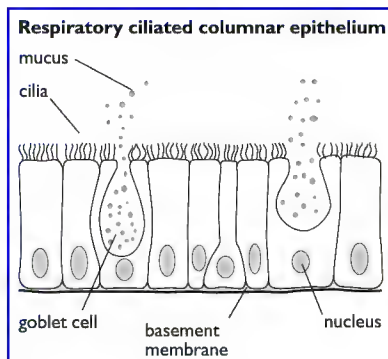
Involuntary (smooth) muscle found in the trachea, bronchi and bronchioles also helps maintain the shape of the tubes and prevents collapse of the airways. The smooth muscle receives nerve impulses from the autonomic nervous system which can regulate the diameter of the tubes. This is significant in diseases like asthma and anaphylaxis where constriction of the bronchioles occurs restricting air flow.

Epithelium lining the trachea, bronchi and bronchioles is known as the mucous membrane, and consists of ciliated columnar epithelium. This possesses: ciliated cells and goblet cells.

Ciliated epithelial cells have a protective function as they work in a co-ordinated fashion sweeping the mucus coat of the air passages, which contains small inhaled particles such as dust, towards the pharynx (throat) where they can be swallowed. This prevents such particles from entering the alveoli and interfering with gaseous exchange. **Goblet cells** are scattered among the ciliated cells in the trachea, bronchi and bronchioles, and are responsible for producing and secreting mucus within the respiratory tract. The layer of mucus which lines the airways traps dust and bacteria.

Alveoli or air sacs make up the mass of the lungs. The ends of the bronchioles terminate in tubes, only 1-2mm in diameter, known as alveolar ducts which lead to the small chambers used for gas exchange (alveoli). Although each alveolus is very small, the large numbers of alveoli (around 700 million) provide an enormous surface area for gas exchange: something in the region of 70-90m² in an adult lung.

Blood vessels of the pulmonary circulation supply the alveoli. Deoxygenated blood enters the lung via the pulmonary artery which initially splits into right and left branches and then follow the airways, branching through the tissue of the lung.



The exchange of respiratory gases in the lungs

Gas exchange occurs by diffusion between the air in the alveoli of the lungs and the blood in the capillaries. The rate of diffusion of a substance is dependent upon the difference in its concentration in two separated regions (the concentration gradient), the distance separating them, and the surface area, a relationship expressed in the following equation known as **Fick's law** (see 10.3).

$$\text{Rate of diffusion} = \frac{\text{surface area} \times \text{difference in concentration}}{\text{thickness of the exchange surface}}$$

This gives a rough guide to the speed with which oxygen diffuses from the watery lining of an alveolus to the blood in a lung capillary. In principle, the greater the surface area, the greater the concentration difference, and the thinner the separating layers, the more rapid the diffusion.

The relative amounts of oxygen and carbon dioxide in the air breathed in and out are measured as 'partial pressures' in units of kiloPascals (kPa).

The partial pressure of a gas is a better measure of the amount of gas present than its percentage composition. Although the percentage of oxygen remains the same (21%) in air under different conditions, the **amount** of oxygen does not. At atmospheric pressures less than at sea level e.g. at altitude, there is still 21% oxygen in air, but as the air is less dense there is a smaller amount of oxygen.

Blood entering the capillaries of the alveoli from the pulmonary (lung) arteries has a lower oxygen and a higher carbon dioxide content, than the air in the alveoli. Therefore carbon dioxide diffuses out of the blood into the alveoli, and oxygen diffuses into the blood from the alveoli, each down their partial pressure gradients.

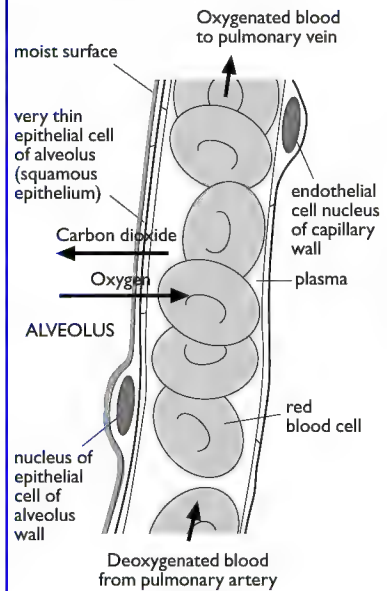
The oxygen diffusing into the blood dissolves in and diffuses through the film of moisture on the surface of the alveoli, through the thin walls of the alveoli, through the thin wall of the capillaries, through the plasma, and through the red blood corpuscle membrane to combine with the haemoglobin to form oxyhaemoglobin.

About 250 cm³ of oxygen per minute diffuses in over the alveoli at rest, and this can be increased up to about twenty times during exercise.

Carbon dioxide diffuses in the opposite direction to the oxygen, from the blood plasma into the alveoli.

Carbon dioxide molecules are larger than those of oxygen and therefore diffuse more slowly in the gas phase, but carbon dioxide is much more soluble than oxygen and therefore diffuses about twenty times more readily in solution than oxygen across the capillary-alveolar barrier. This explains why, although the pressure gradient of carbon dioxide is only 6 mm Hg compared to 60 mm Hg for oxygen, sufficient exchange of CO₂ still occurs.

Gas exchange alveolus/capillary



◆ CHECKPOINT SUMMARY

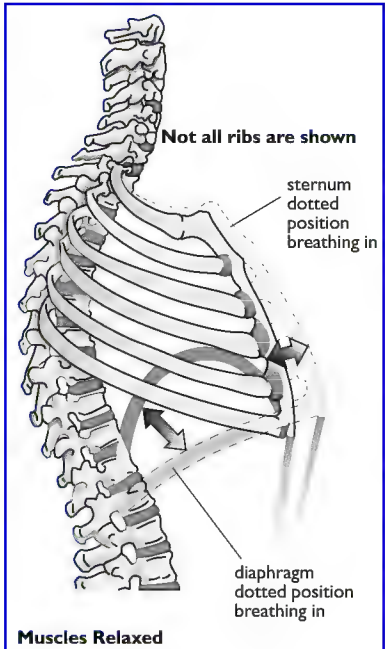
- ◆ Gas exchange occurs over large surface area of alveoli which is a product of their relatively small size and large number
- ◆ Diffusion gradients of oxygen and carbon dioxide dependent upon partial pressures of gases on either side of diffusion surface
- ◆ Diffusion surface composed of thin squamous epithelium ridged around the sandwiched capillaries
- ◆ Surface water an inevitable consequence of a permeable surface exposed to air, decreases diffusion of oxygen more than the diffusion of carbon dioxide
- ◆ Air in alveoli remains fairly constant in composition independent of breathing cycle.
- ◆ Diffusion gradients maintained by circulating blood and ventilation of the lungs
- ◆ As demand for oxygen increases so does steepness of diffusion gradients and speed of circulation thus meeting extra demand.

Exchanges between alveolar air and pulmonary blood at rest

	Blood in pulmonary artery	Alveolar air	Blood in pulmonary vein
	mmHg	mmHg	mmHg
O ₂	40.0	98.0	96.0
CO ₂	46.0	40.0	40.0

Differences between inspired and expired air

	Dry inspired air	Alveolar air	Expired air
	mmHg	mmHg	mmHg
O ₂	159.6	98.0	116.2
CO ₂	0.3	40.0	28.5
N ₂	600.0	575	567.5



VENTILATION

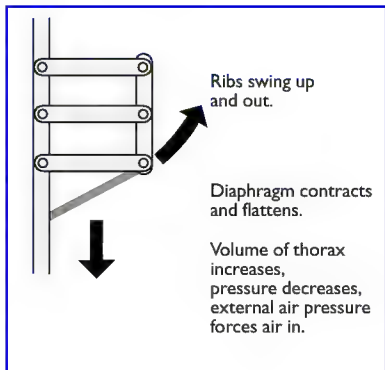
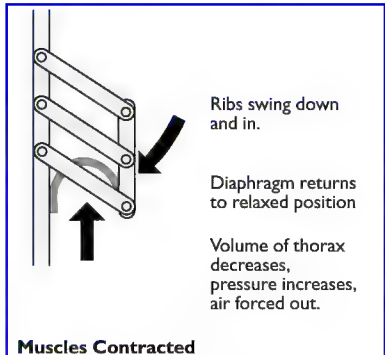
The mechanism of ventilation and its nervous control

For efficient gaseous exchange at the alveolar surface, air must be moved in and out of the lungs in the process called **ventilation**. This is achieved by the movements of the **intercostal muscles** of the ribs and the muscular **diaphragm**, which are controlled by the autonomic and voluntary nervous systems.

During **inspiration** (breathing in) the external intercostal muscles contract pulling the ribs upwards and outwards. At the same time the diaphragm contracts, moving downwards. Both movements increase the volume of the thorax, reducing the pressure in the thorax below atmospheric pressure causing air to enter, inflating the lungs. At rest, **expiration** (breathing out) does not involve active muscle contractions, but depends upon the elasticity of the tissues of the lungs and thorax. The external intercostal muscles relax, allowing the rib cage to move downwards and inwards, and the diaphragm relaxes and regains its domed shape. The volume of the thorax is reduced, pressure increased above atmospheric pressure and air is forced out of the lungs. During forced breathing, for example as a result of exercise or in speech, expiration is active, with the internal intercostal muscles and abdominal muscles contracting to increase the pressure within the thorax.

Friction between the lungs and the inner surface of the wall of the thorax is reduced by the **pleural fluid** which lies between the two pleural membranes that surround the lungs.

Breathing movements result in certain volumes of air passing into and out of the lungs.



The role of the medulla and phrenic nerves in generating a basic breathing rhythm

Normally, respiratory movements are involuntary, being controlled by rhythmic discharges of nerve impulses from the respiratory control centres in the **medulla** region of the brain. From the inspiratory control centre impulses travel down the **phrenic nerves** to the diaphragm, and down the **intercostal nerves** to the external intercostal muscles causing them to contract and bring about inspiration. When the alveoli are stretched at the end of inspiration, stretch receptors in the bronchioles send impulses to the **inspiratory control centre** in the medulla, which is switched off, and the diaphragm and external intercostal muscles relax. The elasticity of the tissues of the lungs and thorax leads to expiration as they recoil. Normally, expiration is passive but it may be assisted by nerve impulses originating in the **expiratory control centre** in the medulla which cause the internal intercostal muscles to contract.

The inspiratory control centre and expiratory control centres are antagonistic to each other, each inhibiting the activity of the other so that neither can operate at the same time. The two centres are responsible for maintaining the basic rhythm of breathing.

The rate of respiration is mainly dependent upon the carbon dioxide concentration of the blood, which has a direct effect on the respiratory centres of the medulla. Any increase in carbon dioxide levels is also detected by sensory receptors (chemoreceptors) located in the aorta and carotid arteries which inform the medulla of the need to increase the rate and depth of breathing. These sensory messages are backed up by other receptors which inform the medulla of increases in temperature and decreases in oxygen levels and blood pH.

◆ CHECKPOINT SUMMARY

- ◆ For efficient gaseous exchange at the alveolar surface, air must be moved in and out of the lungs in the process called ventilation
- ◆ Inspiration (breathing in) - the external intercostal muscles contract, pulling the ribs upwards and outwards, the diaphragm contracts, moving downwards, the volume of the thorax increases, the pressure in the thorax decreases below atmospheric pressure causing air to enter, inflating the lungs
- ◆ Expiration (breathing out) depends upon the elasticity of the tissues of the lungs and thorax. The respiratory muscles relax, the thorax and lungs recoil as a result of their elasticity and the events of inspiration are reversed
- ◆ At sea level the composition of inspired air is relatively constant, but the composition of expired air varies with the activity of the individual
- ◆ More oxygen is consumed and carbon dioxide produced as activity increases.
- ◆ Normally, respiratory movements are involuntary, being controlled by rhythmic discharges of nerve impulses from the respiratory control centres in the medulla region of the brain
- ◆ From the inspiratory control centre impulses travel down the phrenic nerves to the diaphragm, and down the intercostal nerves to the external intercostal muscles causing them to contract and bring about inspiration
- ◆ At the end of inspiration, stretch receptors in the bronchioles send impulses to the inspiratory control centre in the medulla, which is switched off, and the diaphragm and external intercostal muscles relax. The elasticity of the tissues of the lungs and thorax leads to expiration as they recoil.

1.8

The function of the heart in the circulation of blood and in relation to the level of activity of an individual

Contents

Heart structure

- ▼ The gross structure of the heart in relation to its function

Heart function

- ▼ Pressure and volume changes and associated valve movements during the cardiac cycle.
- ▼ Myogenic stimulation of the heart and transmission of a subsequent wave of electrical activity.
- ▼ Roles of sinoatrial node, atrioventricular node and bundle of His.

Effects of exercise

- ▼ Cardiac output as the product of heart rate and stroke volume.
- ▼ Pulmonary ventilation as the product of tidal volume and breathing rate
- ▼ Changes in cardiac output and pulmonary ventilation with exercise
- ▼ Nervous control of heart rate in relation to changing demands.
- ▼ Redistribution of blood flow in response to varying degrees of exercise
- ▼ The relative stability of blood supply to the brain and the increase to skeletal muscle.

HEART STRUCTURE and FUNCTION

The heart is situated in a cavity, slightly to the left of the chest mid-line, protected by the ribs and by a membrane (**pericardial membrane**) which surrounds it in a fluid filled cavity.

The collecting chambers of the heart, the right and left **atria** are situated at the top of the organ, receiving blood from the major veins (**venae cavae** and **pulmonary veins**). Note that diagrams of internal organs are always viewed from the front or anterior side of the body, so that the right atrium and right ventricle appear on the left side of the diagram. The atria are separated from the main pumping chambers (right and left **ventricles**) by flaps of fibrous tissue which act as one way valves, the **tricuspid valve** on the right and the **bicuspid valve** on the left. These atrio-ventricular valves are supported by tendons and muscles which prevent them blowing outwards under pressure. Inside the entrance of the main arteries which leave the heart, the aorta and pulmonary artery are two more sets of valves called **semi-lunar, or pocket valves**, the action of which is described later. Immediately above the semi-lunar valve in the aorta is the entrance to the **coronary artery** which supplies the heart itself with food and oxygen. This branches all over the surface

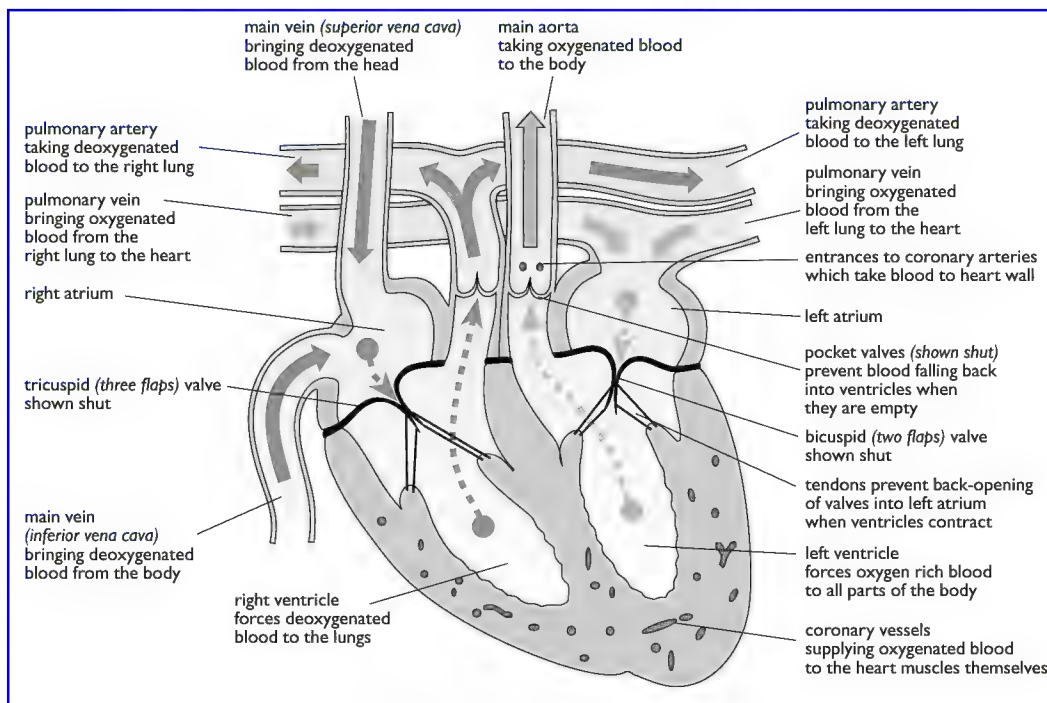
of the heart. Deoxygenated blood is collected into the **coronary vein** which empties directly into the right atrium.

The walls of the atria are less muscular than those of the ventricles, and the wall of the right ventricle is not as muscular as that of the left ventricle. These differences reflect the different amounts of work that the various chambers have to do. The atria only have to pump blood into the ventricles, and the right ventricle pumps blood to the lungs at a lower pressure, than the left ventricle which pumps blood all around the body. However, it is important to remember that the volumes of the cavities of the chambers on the right hand side of the heart are the same as those on the left hand side of the heart, otherwise the right side would not be able to supply the left side with blood.

The blood travels from the heart to the lungs and back to the heart in the **pulmonary circulation**, and then from the heart around the rest of the body and back to the heart in the **systemic circulation**. This arrangement means that the blood can be pumped through the capillaries of the delicate alveoli of the lungs at a lower pressure, than that of the systemic circulation in which a higher pressure is required for the circulation around the body. The lower pressure is a result of the less muscular wall of the right ventricle, and the lower resistance presented by the relative thinness of the elastic walls of the pulmonary arteries, and their arterioles. If the blood pressure was any higher in the pulmonary circulation (as it sometimes is in people suffering from **hypertension** or 'high' blood pressure) the lungs would be at risk of filling with tissue fluid, which would have serious consequences for gas exchange.

◆ CHECKPOINT SUMMARY

- ◆ External appearance of heart includes small 'flap-like' atria, coronary blood vessels, fat deposits, and major vessels entering and leaving
- ◆ Internal structure of two atria and two ventricles. Atria separated from ventricles by flap valves
- ◆ Chordae tendinae and muscles preventing back-opening of bicuspid and tricuspid valves when ventricles contract (ventricular systole)
- ◆ Pocket valves at base of pulmonary artery and main aorta prevent backflow of blood into ventricles when they relax (ventricular diastole)
- ◆ Atria walls thinner than ventricle walls
- ◆ Left ventricle wall thicker than right ventricle wall to pump blood around body (systemic circulation)
- ◆ Right ventricle wall thinner as only has to pump blood via pulmonary circulation around lungs, where reduced pressure prevents formation of tissue fluid in alveoli
- ◆ Volumes of both left and right ventricles the same as right ventricle supplies the left ventricle with blood via the lungs and left atrium.



The cardiac cycle

The sequence of events that occurs during the filling and emptying of the heart is known as the cardiac cycle. At a rate of 70 beats per minute (bpm) the complete cycle takes about 0.86 seconds. It is a continuous sequence of events, but for the purposes of explanation it is convenient to consider it as occurring in a series of stages. There is a point in the cardiac cycle when all the heart valves are shut, which can be taken as the starting point of the cardiac cycle.

Blood returning under low pressure in the main veins (superior and inferior venae cavae) from the upper and lower body enters the right atrium, and at the same time blood returning from the lungs in the pulmonary veins enters the left atrium.

The atria fill with blood, and the rising pressure of the blood in the atria pushes the tricuspid and bicuspid valves open, and both ventricles begin to fill. As the heart fills with blood both atria contract (**atrial systole**) forcing even more blood into the ventricles. These are now stretched, and they both contract (**ventricular systole**) forcing the blood upwards. This upsurge of blood forces the tricuspid and bicuspid valves shut. These valves are prevented from back-opening into the atria by tough tendon cords, which are held taut by contraction of the papillary muscles to which they are attached. The closing of these valves makes the first heart sound (described as 'lub').

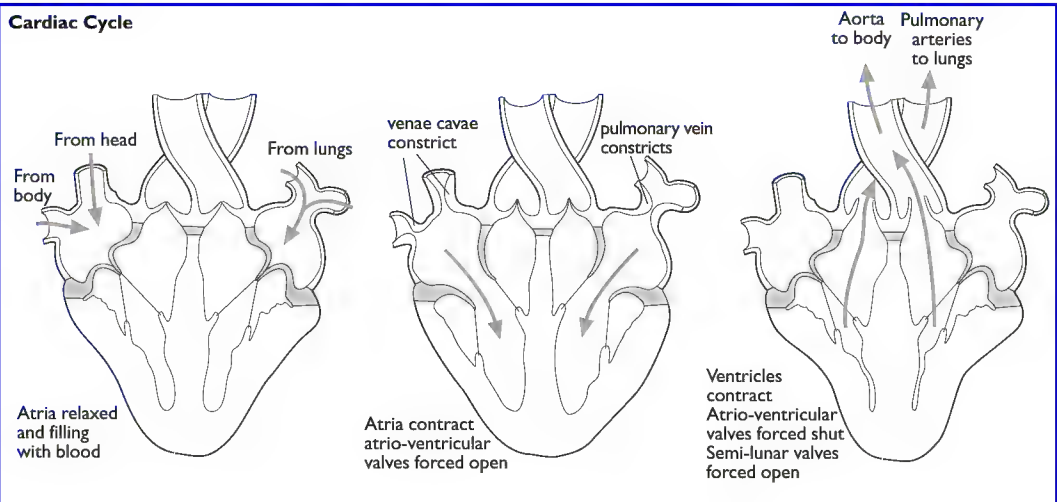
The blood is thus forced along the pulmonary artery to the lungs, and along the main aorta to the rest of the body, forcing open both sets of pocket or semi-lunar valves on the way.

When the ventricles relax (**ventricular diastole**) the blood tends to fall back down the pulmonary artery and main aorta under gravity, thus filling and shutting the pocket valves. The closing of these valves makes the second heart sound (described as 'dub'). All valves are now shut and the cycle is repeated.

◆ CHECKPOINT SUMMARY

- ◆ The events of a complete filling and emptying of the heart are known as the cardiac cycle
- ◆ It is a continuous cycle the start point of which can be taken when heart is empty and all valves shut
- ◆ Atria fill and internal pressure rises forcing bicuspid and tricuspid valves open
- ◆ Ventricles fill so that whole heart full
- ◆ Atria contract (atrial systole) so that ventricles overfilled and stretched
- ◆ Ventricles contract with a force proportional to their degree of stretch (ventricular systole)
- ◆ Blood forced up shutting bicuspid and tricuspid valves (first heart sound) and opening pocket valves as blood forced up pulmonary artery and aorta
- ◆ Ventricles relax (ventricular diastole) blood falls back in pulmonary artery and aorta shutting pocket valves (second heart sound).

Cardiac Cycle



Initiation and control of heart action

Cardiac muscle is **myogenic**, that is it can contract without nervous stimulation. Coordinating waves of electrical activity originate in a mass of specialised cardiac muscle in the right atrium, the **sinoatrial node** (SA node or 'pacemaker'). This provides the basic rhythm of the heart contractions.

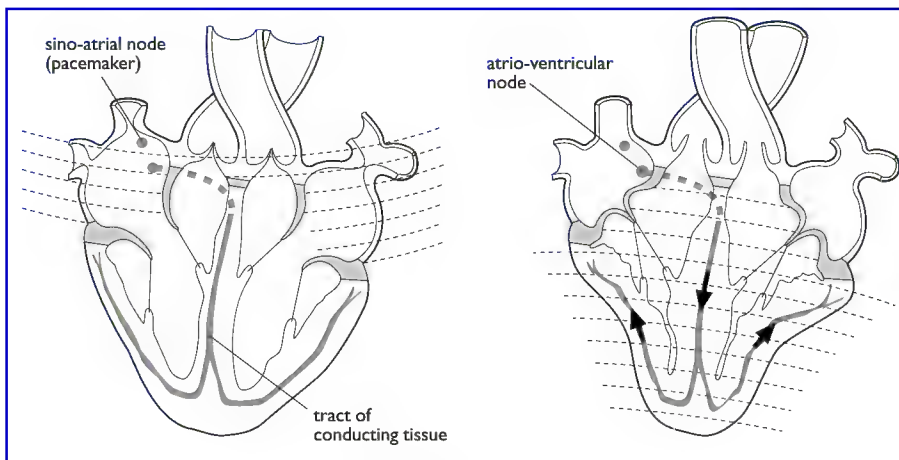
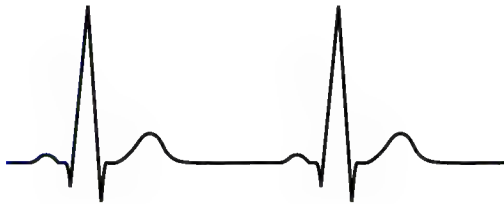
The wave of excitation spreads rapidly through the interconnected cardiac muscle fibres of the atria. However a band of fibrous connective tissue between the atria and ventricles prevents the wave of excitation spreading down into the ventricles. Another mass of specialised tissue, the **atrio-ventricular node** at the bottom of the atria is stimulated, and impulses are transmitted to the base of the ventricles in a tract of conducting tissue (modified cardiac muscle fibres and neurones) called the **bundle of His** which branches into two. The impulses radiate upwards through the cardiac muscle of the ventricles.

This pattern of the spread of excitatory waves through the heart, ensures that the atria contract to force the blood down into the ventricles, and that the ventricles contract to force the blood up into the pulmonary arteries and main aorta. The spread of the wave of excitation throughout the heart can be detected by electrodes attached to the skin of the chest and displayed as an electrocardiograph (ECG trace).

◆ CHECKPOINT SUMMARY

- ◆ Cardiac muscle is myogenic
- ◆ Rate modified by sinu-atrial node (pacemaker) in wall of right atrium
- ◆ Waves of electrical activity spread rapidly down through cardiac muscle of atria
- ◆ Prevented from passing directly into ventricles by barrier of connective tissue, stimulus picked up by atrio-ventricular node
- ◆ Bundle of His composed of Purkyne tissue transmits stimulus to base of ventricles from where it radiates up through the walls back to the connective tissue barrier
- ◆ Detected by electrodes on the skin as an ECG
- ◆ Pacemaker influenced by sympathetic (faster) and parasympathetic (slower) nervous systems, and the hormone adrenaline
- ◆ All influences on heart rate coordinate heart rate and therefore blood supply to body (cardiac output) with demands of body.

ECG trace of electrical activity of heart



EFFECTS of EXERCISE

Cardiac output

The heart pumps blood around a complete circuit of vessels, which offer resistance to the flow of blood, therefore pressures are generated within the system. Blood pressure is usually measured in the brachial artery in the left arm using a sphygmomanometer. It is measured in mm of mercury (mm Hg). As a result of the action of the heart there are two measures of blood pressure.

Systolic blood pressure is that pressure generated when the ventricles are contracting (about 120 mm Hg in a young healthy adult). A rough estimation of systolic blood pressure can be derived from adding 100 to your age.

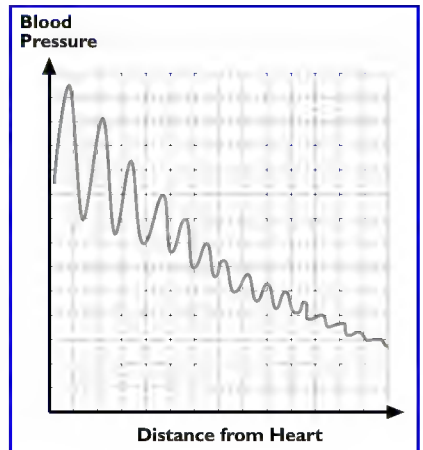
Diastolic blood pressure is that pressure when the ventricles are relaxing (about 80 mm Hg in a young healthy adult). With regard to checking blood pressure, the diastolic pressure gives the clearest indication of the state of the resistance offered to flow by the blood vessels (peripheral resistance), as the heart is not contracting at this time.

Blood pressure is expressed as a ratio of systolic to diastolic blood pressure e.g. 120/80 mm Hg.

The average of the systolic and diastolic pressures during a complete cardiac cycle, known as the **mean blood pressure**, determines the overall rate of blood flow through the circulation. The blood pressure drops as the blood gets further away from the left ventricle and circulates around the body. Some typical figures for the mean blood pressure (in mm Hg) whilst at rest are: arteries 100, arterioles 60, capillaries 18, veins 7, and at the entrance to the right atrium 3.

Blood pressure is generated by the **cardiac output** (heart rate $\times$ stroke volume) and the resistance to flow of the blood in the blood vessels. The resistance is often referred to as the peripheral resistance, as the major part of it occurs in the smaller vessels (mainly the arterioles) at a distance from the heart. The resistance to flow is due to the friction between the blood and the walls of the blood vessels. The friction is determined by the length, diameter, and the smoothness of the lining of the vessels; and the viscosity of the blood. The shorter the vessel, the larger the diameter and the less viscous the blood the lower the resistance. These features, particularly vessel diameter, are not fixed in an individual but change according to circumstances. Both acute (short term) and chronic (long term) changes can occur.

Arteries and arterioles (and to a lesser extent veins and venules) have involuntary muscle fibres in their walls, which when contracted constrict the vessel reducing its diameter. This can lead to short-term (acute) rises in blood pressure and can happen at times of emotional stress, or following intake of certain drugs including nicotine. The systolic and diastolic blood pressures are raised considerably during static (isometric), straining type exercises, as in weight training. This is caused by compression of the peripheral blood vessels, causing an increase in the resistance to blood flow and a demand for increased blood pressure, to maintain the circulation to the contracted muscles. Acute increases in blood viscosity, which lead to increased resistance and increased blood pressure can occur during periods of dehydration due to excess water loss in sweating, or through urine following consumption of substances that increase urine production (diuretics) such as alcohol and caffeine.



Pulmonary ventilation

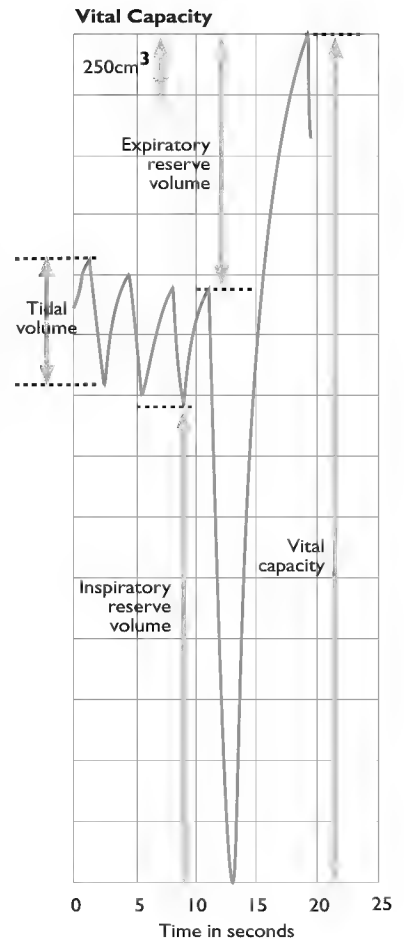
Pulmonary ventilation is the product of the breathing rate (number of breaths per minute) and the tidal volume. The **tidal volume** is the volume of air breathed in and out in a single breath in a normal breathing cycle. A healthy, active mature male of average height would have a tidal volume of about 500 cm^3 . Of this 500 cm^3 , about 350 cm^3 reaches the alveoli where gaseous exchange occurs. The remaining 150 cm^3 fills the pharynx, larynx, trachea, bronchi, and bronchioles, which compared to the alveoli are poorly supplied with blood capillaries. This space where no gaseous exchange occurs is known as the anatomical **dead space**, and the air that occupies it as the **dead air volume**.

The 350 cm^3 of the tidal volume that reaches the alveoli, mixes with the 150 cm^3 of air remaining in the dead space from the previous exhalation, so that 500 cm^3 of mixed air reaches the alveoli on each inspiration. Here it mixes with the air that remains in the alveoli during quiet breathing, the **stationary air volume**, which can be up to 2500 cm^3 . Thus the alveolar air consists of about 350 cm^3 of fresh air mixed with up to 2500 cm^3 of air that has undergone gaseous exchange with the blood, and 150 cm^3 of previous dead space air. Therefore the composition of alveolar air does not fluctuate widely during the breathing cycle, which in turn ensures that gaseous exchange with the blood also remains fairly constant throughout the breathing cycle. However, when the depth of breathing increases during exercise more 'fresh air' does reach the alveoli.

By breathing in as deeply as possible an extra volume of air, in addition to the tidal volume and the stationary air, can be taken into the lungs. This is the **inspiratory reserve volume** and can be up to 2000 cm^3 . By breathing out as forcibly as possible after returning to normal breathing, it is possible to expel some of the stationary air in addition to the tidal volume. This is known as the **expiratory reserve volume** and can be up to 1500 cm^3 . However the lungs cannot be emptied entirely, otherwise they would collapse, and this remaining air is known as the **residual volume** which can be up to 1500 cm^3 .

The sum of the inspiratory reserve volume, the tidal volume, and the expiratory reserve volume is described as the **vital capacity**. The vital capacity represents the maximum total amount of air that can be moved into and out of the lungs by one inhalation and one exhalation, and is about 3000 cm^3 - 4000 cm^3 in the adult male.

These volumes and the lung capacities vary considerably between individuals. They are generally smaller in females than in males, due to smaller average body size in females, and correlate closely with body size, height and surface area up to age 25. Smoking and associated lung diseases can considerably reduce lung volumes and capacities.



This trace does not show the total lung volume and residual volume, which are not easily measured. With increasing age the residual volume increases from about 20% of the total lung volume to more than 30%.

Change in cardiac output and pulmonary ventilation with exercise - nervous control of heart rate

At the onset of exercise, control centres are alerted by sensory receptors of the need for an increase in lung ventilation and cardiac output. In the case of the respiratory rate, the roles of the inspiratory and expiratory centres have been described in 10.7. Exercise causes a rise in carbon dioxide levels in the blood and tissue fluids accompanied by an increase in temperature and a decrease in oxygen levels. All of these factors combine to cause a response by the inspiratory centre in the medulla resulting in an increase in the rate of breathing.

Increased cardiac output is initiated by nervous information from various sources including pressure receptors (baroreceptors) located in major arteries and veins, the medulla of the brain and the adrenal glands. During exercise, the heart rate is caused to increase due to stimulation of the sinoatrial node by sympathetic nerves from the medulla of the brain. This response is enhanced by secretions of the hormone adrenaline from the adrenal gland.

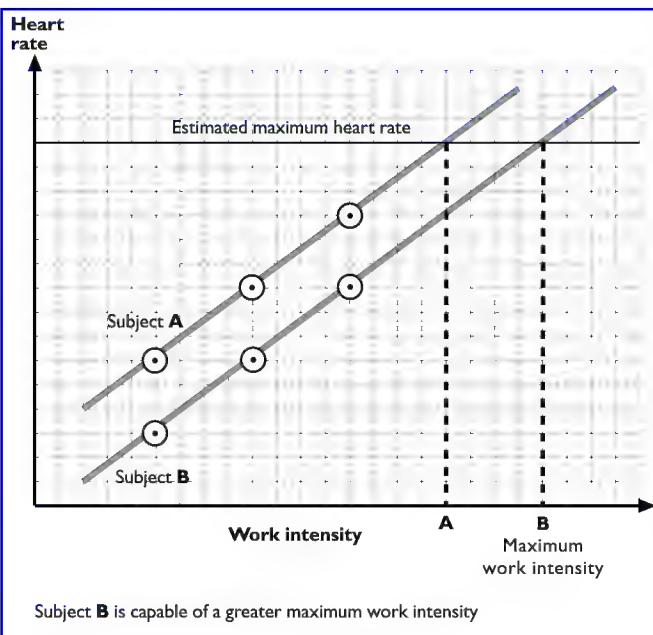
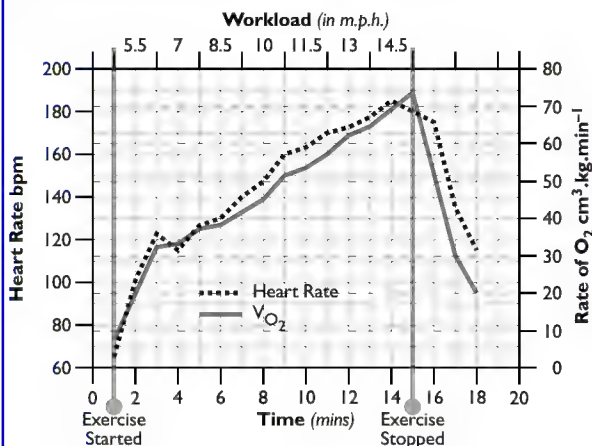
As the heart rate rises during exercise, owing to increased demand for oxygen and glucose by muscles, so too does the pulse rate. A resting pulse rate (heart rate) of around 70 beats per minute can increase to over 200 during vigorous exercise. The maximum heart rate decreases with age, so that an estimate of your maximum heart rate can be given by the result of 220 minus your age.

The heart rate of individuals doing the same exercise will, however, vary considerably. Heart size varies between people, being usually though not always correlated with body size. In general the larger the heart the slower the maximum heart rate, as it takes longer for a larger heart to fill with blood between contractions. It is important to note that this is usually regarded as being beneficial since the rate at which blood is pumped into arteries and hence to tissues (the cardiac output) depends not only on the heart rate, but also on the amount of blood pumped out of the ventricles on each contraction (the stroke volume). The stroke volume is determined by the extent to which the ventricles fill with blood and by the force of contraction of the ventricles which is itself related to this (the fuller the ventricles the greater the force of contraction). Cardiac output is equal to heart rate $\times$ stroke volume. Endurance training over long periods of time can lead to the enlargement of the heart - a condition known as athlete's heart.

The **resting pulse** is defined as the pulse rate measured when an individual is at complete rest. It is usually taken when an individual is lying down first thing in the morning. It represents the response of the heart to basic metabolic demands of the body at rest (mental and physical). In general the larger and more powerful the heart of an individual (perhaps as a result of endurance athletic training) the lower will be the resting heart rate. This is because more blood will be pumped out of their ventricles on each contraction (the stroke volume is larger). Thus for a given cardiac output heart rate is reduced, with some highly trained endurance athletes having resting pulse rates as low as 30 beats per minute.

It can therefore be said that as physical fitness (of the endurance type) increases, heart rate decreases, both at rest and during exercise of a given intensity. This is beneficial as it means that the heart is working less hard and has a lower oxygen demand. Another measure of fitness is the rate at which the heart rate returns to its resting rate after a set period of exercise at a given intensity, the faster the return to normal the greater the physical fitness.

Heart rate and oxygen uptake in response to exercise



CHECKPOINT SUMMARY

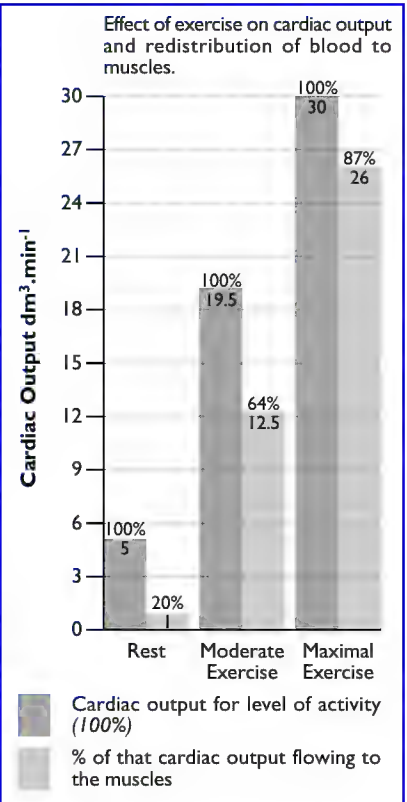
- Cardiac output is the product of heart rate and stroke volume
- Stroke volume is the product of the volume of the ventricle, the degree of filling, and the ejection fraction (the fraction of the blood that it contains that is pumped out)
- Pulmonary ventilation is the product of breathing rate and tidal volume (depth of breathing)
- Both cardiac output and pulmonary ventilation increase with exercise
- Increased cardiac output is initiated by sensory nerve information on increases in blood pressure, carbon dioxide levels in the blood, and decreasing blood pH
- There are so many capillaries in the body that they cannot all be supplied with blood at the same time. Consequently, there is competition for blood between different regions of the body, especially during exercise, when blood must be shunted to the working muscles, and consequently withdrawn from other regions
- For example, when the body is at rest, about 45% of the cardiac output passes through the capillaries of the gut wall and associated glands, and the kidneys
- During maximum exercise this can be reduced to about 3% of the cardiac output, as blood is redirected to the working muscles
- The blood supply to the brain and heart remains relatively stable during exercise, but increases dramatically to the working muscles from about 20 to 88%.

Redistribution of blood flow in response to exercise

There are so many capillaries in the body that they cannot all be supplied with blood at the same time. Consequently, there is competition for blood between different regions of the body, especially during exercise, when blood must be shunted to the working muscles, and consequently withdrawn from other regions. For example, when the body is at rest, about 45% of the cardiac output passes through the capillaries of the gut wall and associated glands, and the kidneys.

During maximum exercise this can be reduced to about 3% of the cardiac output, as blood is redirected to the working muscles. This does not necessarily lead to as large a reduction in oxygen supply to the gut and kidneys as it would seem, as these regions only use about 10 - 25% of the oxygen available in their normal blood supply. The reduced flow rate is compensated for by a greater extraction of oxygen from the blood, by these tissues. However, prolonged reduction in the blood flow to the gut and its associated glands will inevitably interfere with digestion. In short intense periods of exercise the blood flow to the skin is also reduced, even in hot conditions, as it is shunted to the muscles. However, it is increased in longer duration moderate exercise in order to promote heat loss as part of temperature control.

The shunting of blood between competing tissues is achieved by constriction and dilation of the arterioles, and of the arterio-venous vessels, which are direct connections between the arterioles and venules. The entrances to the capillary beds are controlled by circular precapillary sphincter muscles which, when contracted, shut off the capillaries and when relaxed, open them



Estimated blood flow in cm³ per minute, to different organs/systems in a trained male at rest and during maximum effort.

Estimated blood flow in cm ³ per minute				
Organ system	At rest	%	Max. effort	%
Skeletal muscle	1000	20	26 000	88.00
Coronary vessels	250	5	1200	4.00
Skin	500	10	750	2.50
Kidneys	1000	20	300	1.00
Liver & gut	1250	25	375	1.25
Brain	750	15	750	2.50
Whole body	5000	100	30 000	100.00

Biology



Applied Biology

FELTHAM PRESS LTD

Applied Biology

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2.6

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Biotechnology

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2.1

Enzymes in biotechnological processes

Contents

Isolation of enzymes

- ▼ The distinction between intracellular and extracellular enzymes
- ▼ Commercial production of enzymes from microorganisms
 - the growth of large numbers of microorganisms using specific media and septic conditions
 - the isolation and purification of the enzyme product by downstream processing

Application of enzymes in biotechnological processes

- ▼ The applications of enzymes linked to a consideration of their functions
 - enzymes as analytical agents
 - thermostability in industrial processes
 - immobilised enzymes

Introduction

Enzymes are biological catalysts which speed up and co-ordinate chemical reactions in living organisms (see Module 1). They are three dimensional proteins, sometimes with non-protein cofactors attached, whose production is coded for by one or more genes. Several thousands of different enzymes have been identified so far, each catalysing one specific reaction within a narrow range of conditions (e.g. temperature, and pH).

Although enzymes have only been investigated scientifically for just over a century, they have been used by humans for thousands of years in traditional skills, e.g. brewing (the term enzyme means 'in yeast'), and cheese making. While we are still far from identifying and understanding the full range of enzymes and enzyme functions in living cells, knowledge of some enzyme catalysed reactions has led to the development of enzyme-based technology. A range of enzymes now have important commercial applications in industry; including the food and drink industry, the paper industry, and the pharmaceuticals industry. For a range of financial and biological reasons most of the enzymes used in these industries are obtained from fungi and bacteria.

ISOLATION of ENZYMES

Intracellular and extracellular enzymes

Whilst there are many ways of classifying enzymes, one of the most straightforward is a classification based on whether the enzyme functions within or outside the cell which produces it.

Intracellular enzymes are produced inside cells and work inside cells. Examples include DNA polymerase which is involved in DNA replication in the nucleus, all of the enzymes used in cellular respiration, and all of the enzymes involved in photosynthesis.

Extracellular enzymes are produced inside cells but released from cells to work outside of the cell. The most familiar are the digestive enzymes which are produced in cells of the digestive tract or in cells within digestive organs and released into the lumen of the digestive tract. These include salivary amylase produced by cells in the salivary glands of the mouth, and pancreatic lipase and amylase produced by cells of the pancreas and released into the small intestine. In fungi and many bacteria, extracellular enzymes are secreted onto food materials to allow extracellular digestion. Such enzymes are often of great commercial interest.

Enzymes in industry: advantages

Enzymes are biological catalysts, and have a number of unique properties which distinguish them from inorganic catalysts. These properties, which are listed below, make them ideal for commercial use, especially on grounds of economy.

- ▼ Enzymes are highly specific to one substrate. Specificity ensures that only the desired reactions will take place, leading to the production of a pure product. This is particularly crucial in the pharmaceutical industry where enzymes are used to indicate the presence of one substance within a complex mixture.
- ▼ Enzymes work at low temperatures (usually below 40°C) compared to some inorganic catalysts which need temperatures of over 500°C. This reduces the cost of many processes and also makes them safer. In some industrial processes, however, enzymes that work at temperatures over 60°C are used.
- ▼ Enzymes work at atmospheric pressure whilst some inorganic catalysts will only function at much higher pressures. Again this reduces cost.
- ▼ Enzymes are less toxic than many inorganic catalysts, making them more suitable for a variety of processes, particularly in the food and drink industry.

Enzymes in industry: disadvantages

Enzymes do, however, have some disadvantages compared to inorganic catalysts.

- ▼ Enzymes are proteins with a complex 3D shape upon which their activity depends, therefore their efficiency will be affected if the temperature or pH changes from the optimum. At extreme pHs and high temperatures they are irreversibly denatured.
- ▼ Enzyme action may be inhibited by other substances present in a mixture, including the products of their own reactions.

-
- ▼ The initial stages of research, required to identify suitable and safe enzymes in organisms, are very expensive. In many cases production and isolation of enzymes is also expensive, particularly when they are intracellular enzymes.

Production of enzymes using microorganisms

In the past, enzymes required for use in commercial processes were extracted directly from their source in an animal or plant. Hence chymosin or rennilase (formerly rennin) used in cheese making was taken from the stomach of new born calves. Such methods are however very wasteful and expensive and cannot be used to produce enzymes on a large scale. As commercial demand has grown, methods using enzymes produced by microorganisms have developed. In addition to the significant fact that microorganisms naturally produce many extracellular enzymes, other benefits include:

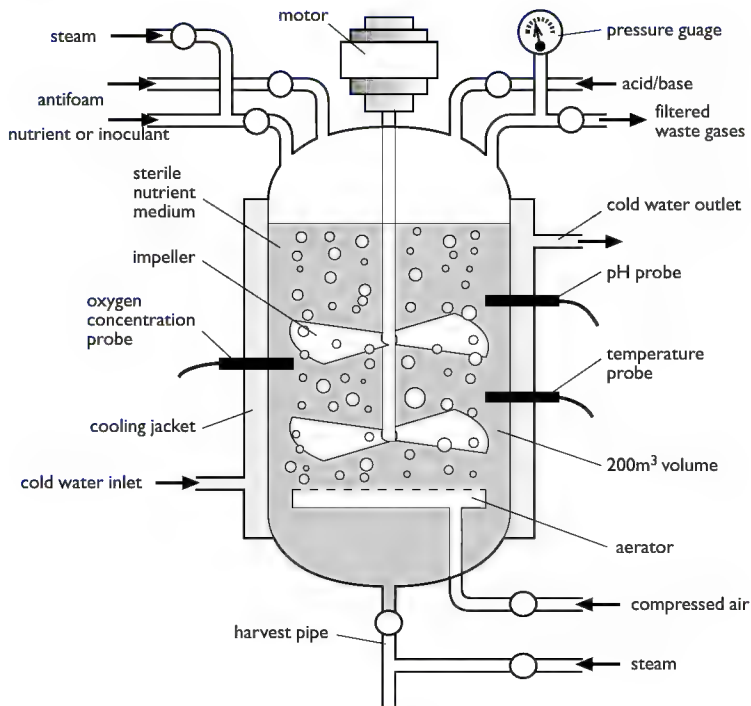
- ▼ If correctly monitored and grown inside specially designed fermenters (see below) such organisms have massive growth rates at low cost.
- ▼ They use only simple nutrient substances or even waste products, contributing to the low costs of the process.
- ▼ They can be easily genetically engineered to produce a range of enzymes originally derived from other species.
- ▼ Mutant strains producing particularly high yields of particular enzymes can often be developed
- ▼ Their exploitation does not raise the same ethical issues associated with the use of animals, e.g. the slaughter of calves to produce chymosin.

Growth of microorganisms

The principles of growing microorganisms in fermenters for the commercial production of enzymes follows the same basic principles as the growth of microorganisms in a laboratory. If optimum growth is to be achieved then the microorganisms require a suitable nutrient medium in which to grow, suitable environmental conditions, and control of competition or predation from other microorganisms.

Nutrient requirements vary between species, but all microorganisms require simple organic sources of carbon and nitrogen (such as simple sugars and amino acids), and a range of vitamins and minerals, dissolved in water. Most species will grow best if this medium is kept at around 35-40°C, and at a pH of close to 7. Oxygen requirements will vary from anaerobic conditions to aerobic conditions. It is also important that the fermentation mixture containing the microorganisms and the nutrient medium is well mixed. The term fermentation used to be restricted to anaerobic conditions, but is now used more generally. Most reactions in 'fermenters' are in fact aerobic.

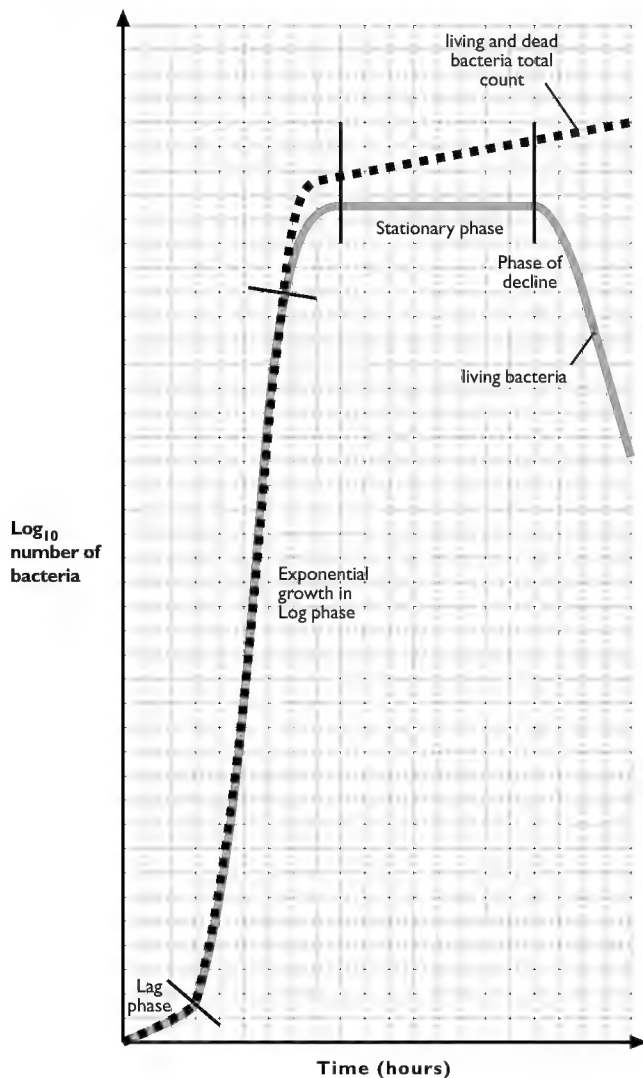
Bioreactor



Given optimal conditions microorganism growth will follow a characteristic pattern. It will begin slowly and then accelerate exponentially. After this point there are two possible scenarios. If the bacteria are left alone, their growth rate will level off and then decline until it crashes to zero, as nutrients and oxygen have been used up, and waste products accumulated. This pattern is used in **batch fermentation** of microorganisms where a single culture of microorganisms and a single addition of nutrient medium is used to produce a batch of enzyme. This is used in almost all cases, producing large batches of commercial enzymes such as chymosin (rennilase) for the cheese industry, and amylase for the baking industry.

In **continuous fermentation** the bacteria are given more nutrients and oxygen, and other conditions are kept optimal, allowing them to continue to grow at a maximum rate. This allows continuous production and extraction of an enzyme. The growth rate of the bacteria can be adjusted by varying the quantity of nutrients added. An example of the use of continuous culture is in the growth of *B. coagulans*, the bacterium which produces glucose isomerase used in the production of high fructose corn syrup.

Growth of microorganisms



◆ CHECKPOINT SUMMARY 30

- ◆ Intracellular enzymes are produced inside cells and work inside cells. Extracellular enzymes are produced inside cells but released from cells to work outside.
- ◆ Growth of large numbers of microorganisms in fermenters for the commercial production of enzymes requires a suitable nutrient medium in which to grow, suitable environmental conditions, and absence of competition from other microorganisms (i.e. sterile conditions).
- ◆ Nutrient requirements vary between species but all microorganisms require simple organic sources of carbon and nitrogen (such as simple sugars and amino acids), and a range of vitamins and minerals dissolved in water.
- ◆ Given optimal conditions microorganism growth will follow a characteristic pattern. It will begin slowly and then grow exponentially.
- ◆ If the bacteria are left alone their growth rate will level off and then decline until it crashes to zero as nutrients and oxygen have been used up.
- ◆ Alternatively, if the bacteria are given more nutrients and oxygen and other conditions are kept optimal they will continue to grow at a maximum rate.
- ◆ It is essential that the fermenter and all pipes leading to and from it are sterile before microorganisms are introduced. This also applies to all substances added to the fermenter during fermentation. Such sterile conditions are described as aseptic.

Bacteria culture at 30°C

Time/h	Number of cells in millions	
	Living	Living & Dead
0	9	10
1	10	11
2	11	12
5	18	20
10	400	450
12	550	620
15	550	700
20	550	850
30	550	950
35	225	950
45	30	950

Aseptic conditions

It is essential that the fermenter and all pipes leading to and from it are sterile before microorganisms are introduced. This also applies to all substances added to the fermenter during fermentation. Such sterile conditions are described as aseptic. If these conditions are not maintained then microorganisms other than those required will also grow inside the fermenter. Since the conditions are, of course, ideal for growth of microorganisms, unwanted or harmful microorganisms can also rapidly multiply. At worst this could contaminate the product with toxins or poisons that could cause harm to consumers. Less serious but still problematic is the fact that unwanted microorganisms could feed on or compete for resources with the useful microorganisms, so limiting productivity.

To ensure aseptic conditions the fermenter and all inlets and outlets are sterilised with steam under high pressure before use. Air entering the fermenter is filtered, and nutrient media may also be heat sterilised. As the culture organisms could also be a source of contamination they are checked for the presence of other microorganisms. Air leaving the fermenter may also be filtered to prevent microorganisms escaping.

Microorganisms which have high optimum temperatures for growth are particularly favoured in biotechnology as this reduces the chance of contamination by other species. The enzymes so produced will also be thermostable, the advantages of which are discussed below.

Isolation of enzymes

Although the majority of enzymes produced by microorganisms are intracellular, the majority of those used in industrial processes are extracellular. This reflects the fact that isolation and purification of intracellular enzymes is usually difficult and expensive.

Extracellular enzymes are secreted from microorganisms into the culture medium and can usually be isolated from this medium with relative ease. Examples include glucoamylase an extracellular enzyme from *Rhizopus* or *Aspergillus* species, which is used in the processing of corn starch, and chymosin (rennilase) from genetically engineered bacteria, and moulds e.g. *mucor* or yeast, which is used in cheese production.

Intracellular enzymes can only be obtained by damaging the cells in which they are produced. Cells may be disrupted mechanically, or by procedures such as freezing-thawing, osmotic shock, the use of high temperatures, high pHs or digestion by other enzymes. Once the cells have been broken up in this way filtration or centrifugation is used to remove the cell debris, and ultrafiltration or chromatography are used to purify the desired enzyme. Examples of important intracellular enzymes obtained in this way include glucose oxidase (used in biosensors discussed below) and glucose isomerase (used in corn starch processing).

◆ CHECKPOINT SUMMARY

- ◆ The majority of enzymes used in industrial processes are extracellular. This reflects the fact that isolation and purification of intracellular enzymes is usually difficult and expensive.
- ◆ Extracellular enzymes are secreted from microorganisms into the culture medium and can usually be isolated from this medium with relative ease.
- ◆ Intracellular enzymes can only be obtained by damaging the cells in which they are produced. Cells may be disrupted mechanically or physically by procedures such as freezing-thawing, osmotic shock, the use of high temperatures, high pHs or digestion by other enzymes.
- ◆ Once the cells have been broken up in this way filtration or centrifugation is used to remove the cell debris and ultrafiltration or chromatography to purify the desired enzyme.

Application of enzymes in biotechnological processes

Examples of commercial applications of enzymes:

- ▼ Dairy products: forms of rennin and rennin substitutes, e.g. chymosin (rennilase) used to cause milk to coagulate in the production of cheese
- ▼ Fruit juices, wines: pectinases used in the removal of pectins and starches from juice and wines to prevent cloudiness.
- ▼ Brewing: amylases and proteases to digest polysaccharides and proteins in beer and to prevent cloudiness.
- ▼ Sugar: use of amylases and other carbohydrases to produce fructose and glucose from corn (maize) starch. In the processing of cane sugar (sucrose) carbohydrases are also used to remove polysaccharides.
- ▼ Meat: proteases (papain) used to tenderize meat by partially digesting protein fibres before sale.
- ▼ Baking: carbohydrases used to weaken gluten and produce soft textured cakes and breads.
- ▼ Additives: emulsifiers and flavourings for foods may be produced using enzymes.
- ▼ Alcohol production (for drinking and for fuel): amylases and other carbohydrases are used to convert unfermentable starches to fermentable sugars which yeast can utilise. In the production of alcohol for fuel the starting point is agricultural waste, particularly straw.
- ▼ Animal feeds: various enzymes are used to improve the nutritional value of foodstuffs.
- ▼ Paper industry: used in biobleaching, extraction of cellulose from wood (biopulping) and even in de-inking of recycled paper.
- ▼ Leather industry: various proteases used to remove hair from hides and to soften leather.
- ▼ Detergents: proteases, amylases and lipases used in 'biological' washing powders break down food stains on clothes. Also cellulases help to digest loose fibres in cotton, preserving the texture and appearance.
- ▼ Pharmaceutical, analytical and medical: a range of enzymes produced for use in testing kits such as the ELISA test, in biosensors (detecting glucose levels), and test sticks for glucose, proteins and uric acid.
- ▼ Waste treatment: various enzymes used to break down wastes including lignin, cellulose and other solid and toxic materials.
- ▼ Genetics: restriction enzymes (endonucleases) are used to cut DNA for use in genetic engineering, genetic fingerprinting. Polymerases are used to amplify DNA (section 11.3).

Enzymes as analytical reagents

Enzymes are ideal tools for use in biochemical analysis in both industry and medicine. They have indeed revolutionised many clinical tests which aim to detect the presence of certain compounds in biological fluids (blood, plasma, urine etc) and are widely used in hospitals and laboratories. They are also used in industrial processes, including the production of ethanol and other materials in fermenters. The two main advantages of enzymes over other analytical reagents are:

- ▼ Enzymes have a high specificity: They will only react with one specific substrate, even if that substrate is found in a complex mixture.
- ▼ Enzymes have a high sensitivity: they can catalyse reactions even when the substrate is present in very low concentrations.

Enzymes are used in three main types of system:

Diagnostic test strips: basic plastic strips containing a pad with an immobilised enzyme(s) (see below for discussion of immobilised enzymes), show a colour change if the substrate is present, and give a semi-quantitative result via a graded colour change. These are very cheap but can only be used once.

Enzyme electrodes or '**biosensors**': contain an immobilised enzyme, a transducer and a tiny electric circuit. The enzyme catalysed reaction produces a product which generates an electric current in the transducer. The current is proportional to the amount of product (and hence substrate) present and is converted into a digital read out. These are small and reusable tools.

Enzyme analytical reactors: Large scale laboratory tools using soluble enzymes and colour changes to test hundreds of samples per day.

Examples of enzymes in enzyme electrodes used in medicine and industry:	
Enzyme(s)	substrate/anylate
Alcohol dehydrogenase:	alcohol
Cholesterol esterase and cholesterol oxidase	cholesterol
Glucose oxidase and peroxidase	glucose
Urease	urea

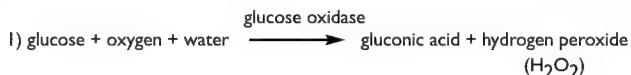
Enzyme(s)	substrate/anylate
Alcohol dehydrogenase:	alcohol
Cholesterol esterase and cholesterol oxidase	cholesterol
Glucose oxidase and peroxidase	glucose
Urease	urea

Using enzymes to test for glucose

One of the most common uses of enzymes as analytical reagents in hospitals is in tests for blood glucose. The level of glucose in the blood is closely regulated in a homeostatic fashion by the pancreatic hormones insulin and glucagon. The normal level is around 90-150mg/100cm³ of blood. Both high and low levels of glucose are harmful and are useful indicators of disease. In particular very high levels of glucose in the blood following meals are characteristic of the disease diabetes. This disease is also characterised by the presence of glucose in the urine. Glucose levels in blood and urine can be detected using the two enzymes: glucose oxidase and peroxidase.

▼ Diagnostic test strip: for testing glucose levels in urine

The colourless pad on the test strip contains the enzymes glucose oxidase and peroxidase immobilised in a cellulose mat along with a colour reagent (e.g. chromagen). When the pad is dipped into a substance containing glucose the reactions shown below occur. The end point is a blue colour (resulting from the reaction of the hydrogen peroxide with chromagen). The shade of blue reflects the concentration of glucose present and is thus semi-quantitative. The pad will only take about 15 seconds to change colour.



Test strips of this kind are used regularly by diabetics to check their urine for the presence of glucose. A positive result would indicate that their blood glucose level was too high. Other simple test strips are available to test blood or urine for a range of compounds including urea (using urease enzyme), and cholesterol (using cholesterol esterase and cholesterol oxidase).

▼ Glucose testing using a biosensor

The biosensor uses the immobilised enzyme glucose oxidase. If a substance containing glucose is placed on the test area of the biosensor the reaction noted above takes place. The gluconic acid produced by the reaction generates an electric current in the transducer which is translated into a digital read-out. The read-out takes less than 20 seconds to appear and gives a quantitative measure of the amount of glucose present in the sample. This method requires a very small sample, a drop of blood from a pin prick is adequate.

Biosensors like this, which are easy to use, relatively cheap yet also highly sensitive and specific, are now commonly used in hospitals to measure the levels of a range of biologically important substances in body fluids.

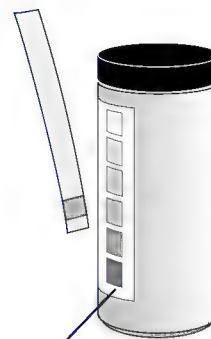
Immobilisation of enzymes

Since enzymes, like all catalysts, are not used up in the processes they catalyse they can, potentially be reused again and again. This is particularly desirable since enzymes are expensive to produce. One of the difficulties of reusing enzymes is, however, the difficulty of recovering the enzyme from the substrate/product where it is in a very low concentration. This can be a time consuming and expensive exercise. If the enzyme is not recovered then not only is the process wasteful, but it may also be harmful as the final product will be contaminated with enzyme.

Testing for glucose using a diagnostic strip



enzyme and chromagen impregnated pad



analysis made by comparison with colour chart on container

For these reasons the development of immobilised enzymes has improved enzyme technology enormously. Immobilised enzymes are enzymes that have been attached to another (insoluble) material in some way and hence prevented from moving freely within the substrate mixture. They may be physically immobilised within a system by being attached to a fixed membrane or gel made of a range of substances including silica, starch, and cellulose. Processes used to attach the enzymes are adsorption, covalent bonding, and entrapment. In other cases the enzyme may move within the substrate but only once encapsulated within beads of another material. In the 'attached' systems enzymes never become mixed with the product, whereas encapsulated enzymes are mixed in with the final product but are easily extracted by filtering or other methods.

There are many advantages to immobilising enzymes, in addition to cost and ease of extracting a pure product.

- ▼ Immobilised enzymes may be more easily added to and removed from reactors to allow the rate of reaction to be more carefully controlled. This means that enzymes can be used to partially, but not completely, digest a substrate.
- ▼ Immobilised enzymes can lead to much greater production efficiency. Primarily they can be used in a continuous enzyme reactor where substrate is constantly added, and product constantly produced, without the need to add or extract enzyme. The best example of this is in the production of high fructose corn syrup described below.
- ▼ Immobilised enzymes tend to be more stable. In particular they are less likely to be inactivated or denatured by high temperatures (they are more thermostable), e.g. glucose isomerase discussed below is stable at 65°C when immobilised but is denatured at 45°C when free; by changes in pH, or the presence of other chemicals.
- ▼ Immobilised enzymes can also be used for longer before their activity decreases, and are less likely to lose activity during periods of storage.

A disadvantage of immobilising enzymes is that the rate of reaction may be slower because the enzyme has reduced kinetic energy and is often partitioned from the substrate by a membrane through which the substrate must diffuse.

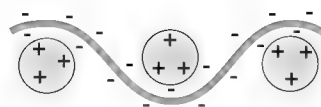
The importance of thermostability

The continued profitability of the 'high fructose corn syrup' (HFCS) industry and other industries relying on enzyme based reactions has depended on the discovery of increasingly thermostable (heat tolerant) enzymes which can be used at temperatures up to and above 60 °C. Many of these enzymes come from bacteria which inhabit hot springs or live deep in the earth's core, where enzymes tolerant of temperatures up to 250°C have been discovered. Enzyme thermostability is an essential feature of such industries for the following reasons:

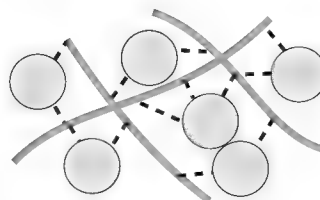
- ▼ The higher the temperature, the lower the chance of contamination with micro-organisms which are generally inhibited at 60°C.
- ▼ The higher the temperature the faster the rate of reaction. This makes the plant more efficient, as more substrate can be processed in a given time.

Immobilised enzymes-different systems

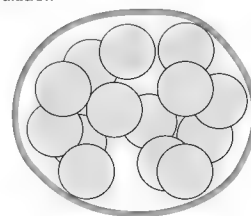
Absorption



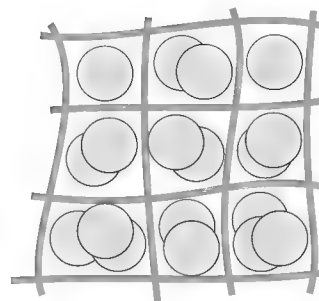
Covalent bonding



Encapsulation



Entrapment



- ▼ If the enzyme is thermostable then it may allow the same enzyme to be used for more batches before its activity declines.
- ▼ The higher the temperature the less viscous the substrate. This is an important factor in the production of HFCS. A very viscous substrate is difficult to stir and thus it will take longer for enzymes to come into contact with the substrate. This is important in the first two stages of HFCS production. The higher the temperature the higher the solubility of reactants. Again this speeds up the reaction, improving productivity.

Corn starch processing: the importance of thermostable and immobilised enzymes

The digestion of corn (maize) starch to produce high fructose corn syrup (HFCS) is one of the largest scale uses of enzymes in industry. In the USA several HFCS plants each produce over 1 million tonnes of the syrup per year. The process is totally reliant on enzymes, and involves three main stages:

- ▼ **Thinning** of the viscous corn starch using amylase enzyme. This stage is carried out at a temperature of 105°C using enzymes from *Bacillus stearothermus* or *B. licheniformis*. The high temperature is particularly valuable in decreasing the viscosity of the mixture.
- ▼ **Hydrolysis** of the pre-thinned starch (dextrins) to a glucose syrup using glucoamylase, an extracellular enzyme from *Rhizopus* or *Aspergillus* species. This enzyme is not immobilised but used as a soluble enzyme in batches at temperatures of 55-60°C. The main reason for this is that the reaction is faster when the enzyme is not immobilised. Also as the enzyme is very cheap, the need for new batches of enzyme each time is not a major expense. The use of batches also allows production to be monitored more easily. Afterwards the product is filtered and passed through an ion exchange mechanism to remove the enzyme.
- ▼ **Conversion or isomerisation** of glucose syrup to fructose syrup (HFCS). Fructose is much sweeter than sucrose or glucose so it is a more valuable product. This stage, leading to the production of syrup containing about 55% fructose is performed by the immobilised enzyme glucose isomerase. This enzyme is attached in various ways to the interior of large columns through which the glucose syrup is continually passed. Fructose syrup (HFCS) continuously emerges from the column in a relatively pure form as it is not contaminated with enzyme. This occurs at 60°C as the enzyme used is not very thermostable.

'Biological' detergents

Enzymes which are stable at higher temperatures are also valuable in the production of biological washing powders. Such powders use proteases, lipases, amylases and cellulases to aid the removal of food and other stains from clothes. Although some early forms used enzymes that could only resist temperatures of 40°C, newer products can be used in washes at 60°C or above with improved results.

◆ CHECKPOINT SUMMARY

- ◆ Enzymes are ideal tools for use in biochemical analysis in both industry and medicine. They have a high specificity and will only react with one specific substrate, even if that substrate is found in a complex mixture.
- ◆ Enzymes have a high sensitivity: they can catalyse reactions even when the substrate is present in very low concentrations.
- ◆ Enzymes are used in three main types of system: diagnostic test strips: enzyme electrodes or 'biosensors': enzyme analytical reactors.
- ◆ Industrial processes require a high degree of thermostability (heat tolerance).
- ◆ The higher the temperature the lower the chance of contamination with micro-organisms, the faster the rate of reaction, the less viscous the substrate and the higher the solubility of reactants.
- ◆ Immobilised enzymes are enzymes that have been attached to another (insoluble) material in some way and hence prevented from moving freely within the substrate mixture. They may be physically immobilised within a system by being attached to a fixed membrane or gel made of a range of substances including silica, starch, and cellulose.
- ◆ Immobilised enzymes may be more easily added to and removed from reactors to allow the rate of reaction to be more carefully controlled.
- ◆ Immobilised enzymes can lead to much greater production efficiency. Primarily they can be used in a continuous enzyme reactor where substrate is constantly added and product constantly produced without the need to add or extract enzyme.
- ◆ Immobilised enzymes tend to be more stable (better able to resist alteration to shape and activity.) In particular they are less likely to be inactivated or denatured by high temperatures (they are more thermostable), changes in pH, or presence of other chemicals.
- ◆ Immobilised enzymes can also be used for longer before their activity decreases and are less likely to lose activity during periods of storage.
- ◆ A disadvantage of immobilising enzymes is that the rate of reaction may be slower because the enzyme has reduced kinetic energy and is often partitioned from the substrate by a membrane through which the substrate must diffuse.

2.2

Cell division

Content:

Mitosis

- ▼ The process of mitosis emphasising the behaviour of chromosomes, the role of the spindle and the genetic identity of the products.
- ▼ The use of appropriate staining techniques in the study of mitosis in suitable plant material (see practical work).

The cell cycle

- ▼ Mitosis and the cell cycle.
- ▼ The relationship between DNA replication and the events of the cell cycle.

Meiosis

- ▼ The importance of meiosis in halving the chromosome number in gametes so that, after fertilisation, the diploid chromosome number is restored in the resulting zygote.

MITOSIS

New cells (daughter cells) are formed by cell division. Cell division is initiated by the nucleus, and nuclear division is the first visible sign of the process of cell division. Mitosis is the commonest form of nuclear division, and results in two genetically identical daughter nuclei being formed. Mitosis occurs in the cell division seen in growth, repair, the replacement of cells which have a limited life span like our red blood cells and skin cells, and in asexual reproduction in many plants and lower animals. In the case of single celled organisms this type of asexual reproduction is called binary fission. The development of a multicellular organism from a fertilised egg by mitosis, means that all the cells of the body are genetically identical to the fertilised egg. The development of an organism from the fertilised egg is the story of the controlled expression of this genetic information, with only a small fraction of the genes being expressed in cells as they differentiate to form tissues specialised for specific (and limited) functions. In mature cells most of the DNA is inactive all of the time.

Asexual reproduction does not involve the fertilisation of gametes, and as a result of mitosis produces offspring which are genetically identical to each other (clones). Many multicellular organisms, especially plants can reproduce their entire bodies in this way, either from specialised structures, or single celled spores.

The production of genetically identical cells in the growth and development of multicellular organisms allows certain cells to retain the ability to develop into any other type if needed as a result of some damage. For example, stem cells in mammals can divide to form any of the different types of blood cell. In the asexual reproduction of organisms mitosis allows for the rapid exploitation of optimal conditions, where any variation would be disadvantageous.

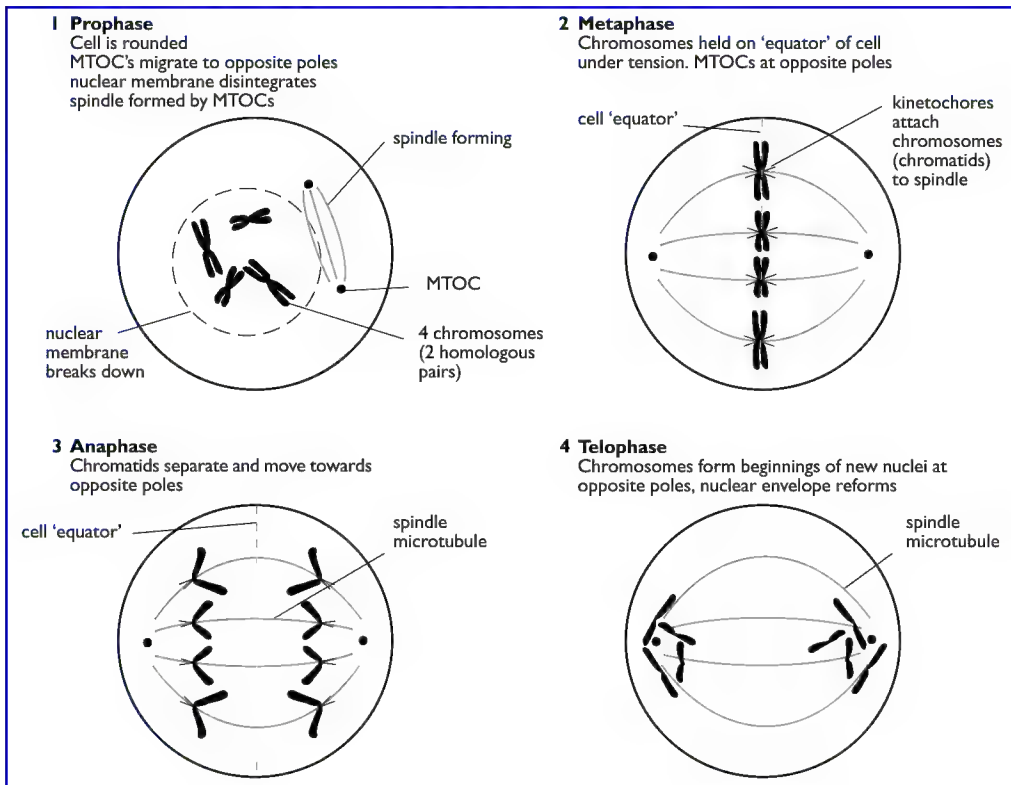
The main stages of mitosis

Chromosomes only form and become visible (when stained and viewed under the light microscope) when the nucleus of a cell is just about to divide. This is because only at this time is the DNA/histone mixture wound up (condensed) as tightly as it can be. By this stage the DNA has also copied itself (replicated) so each chromosome appears as a double structure, joined at one point (the centromere). Whilst they remain joined in this way the two daughter chromosomes are referred to as chromatids.

Nuclear division (mitosis) occurs in four main stages, namely prophase, metaphase, anaphase and telophase.

Prophase is the first phase of mitosis, and as it begins, the DNA has already replicated, all protein synthesis has stopped, and each chromosome is condensed into the characteristic double chromatid form. At the same time the cell rounds up, the nuclear membrane breaks down into small fragments, and a spindle of microtubules forms between two microtubule organising centres (MTOCs) at opposite poles of the cell.

Metaphase is defined as when the chromosomes become attached to the spindle by structures called centromeres (kinetochores). Kinetochores consist of microtubules and 'motor' proteins which utilise ATP energy to pull on the spindle. The effect of both sets of kinetochores pulling in opposite directions is that the chromosomes line up along the middle (equator) of the spindle.



Anaphase occurs when quite suddenly, and all at once, the kinetochores and chromatids of each double chromosome separate, with each chromatid (now daughter chromosome) being pulled under tension in opposite directions towards the poles of the cell by the kinetochores, with their arms trailing in the characteristic 'V' shape.

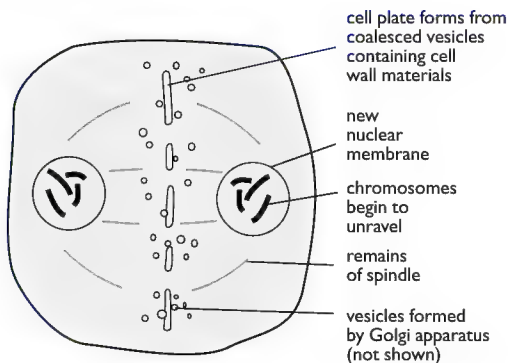
Telophase is the stage when the two new, identical sets of chromosomes reach the opposite poles and a new nuclear envelope forms to surround each set.

Nuclear division by mitosis is followed by division of the cytoplasm into two equal parts. This process is called **cytokinesis** and it occurs differently in animals and plants.

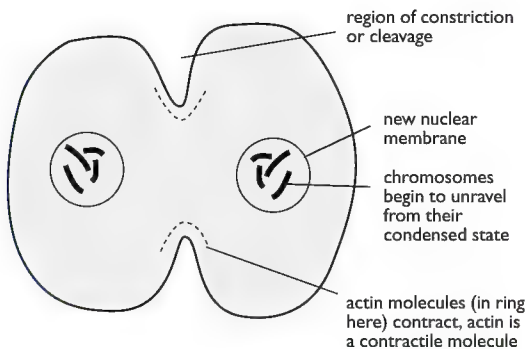
In animal cells the spindle disintegrates during telophase and the microtubule components reassemble into a new cytoskeleton. The equator of the cell is constricted by a ring of contractile proteins (actin) in the process of cleavage, to create two roughly equal cells.

In plant cells the spindle lingers a little longer and seems to act as a guide to bring Golgi apparatus and associated secretory vesicles to the equator. The vesicles deposit their contents, which are the building constituents of a new cell wall, on the equator to form a plate of deposited material (the cell plate). Some of the vesicles remain intact and make connecting channels through the new cell wall (plasmodesmata).

Cytokinesis in plant cells



Cytokinesis in animal cells



THE CELL CYCLE

Interphase refers to the greater part of the life of a cell when there is no apparent activity. The period of mitosis and cytokinesis occupy only a fraction of the entire life of a cell. Together, interphase, mitosis and cytokinesis make up the **cell cycle**. The term interphase should not be interpreted to mean that the cell is resting. Interphase can itself be divided into three distinct periods called G1, S and G2. ('G' refers to the 'gaps' between DNA replication and mitosis.)

G1 is by far the longest period of the cell cycle. During G1, the cell machinery carries out protein synthesis, and growth occurs. All the cells of the body have identical sets of genes in their nuclei but the cells develop in different ways to perform different functions. This development process is called cell differentiation in which different genes are switched on and off, so that only one small part of the genetic code is active in each cell.

After a period of growth and differentiation, the length of which depends upon a variety of internal and external factors, protein synthesis is suspended, and the DNA set replicates itself. This is the **S** phase. DNA replication is followed by another 'gap' period, **G2**, before mitosis starts, in which the cytoskeleton of the cell breaks down and the microtubule components begin to reassemble into spindle parts. Without a cytoskeleton, the cell assumes a more spherical shape (see prophase).

The timing of events within the cell cycle is extremely variable. In ideal conditions, where food and oxygen are in plentiful supply and temperature and pH values are optimal, bacterial cells can complete the cycle to produce a new generation every 20 minutes. Other cells, for example muscle cells, never complete the cycle, a condition called terminal differentiation.

It has become clear that the onset of the S (DNA replication) phase and the M (mitotic) phase are determined by genes which are very similar in all organisms. These genes direct the synthesis of enzymes which in turn activate the enzymes initiating S and M phases.

The rate of cell division can be controlled by naturally occurring plant substances which affect the process of nuclear spindle formation. These include vincristine and vinblastine (from the periwinkle plant *Catharanthus rosea*). Both of these substances are used to control cancers such as leukemia and lymphoma. They work, at a molecular level, by binding on to the spindle microtubules, preventing spindle assembly.

The plant substance, taxol, from the Pacific Yew (*Taxus brevifolia*) has almost the reverse effect on spindle formation, causing an over production of microtubules which effectively stops cell division. Taxol has been used to treat ovarian cancers. As more is known about the factors controlling the cell cycle, new possibilities will open up for drug therapy which can be more specifically targeted. Work in this field will be aided by the research which has discovered the sequence of bases in the DNA of the human chromosomes (the Human Genome Project) as it reveals more and more about the predisposition of individuals with particular gene combinations to cancer.

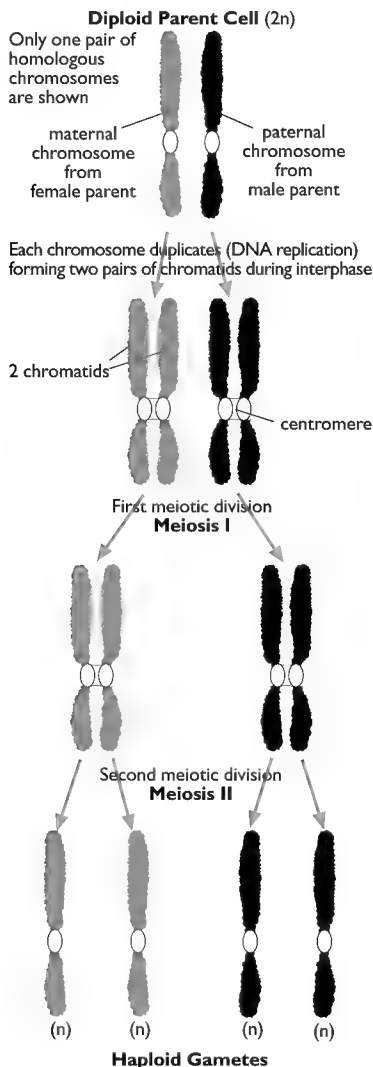
◆ CHECKPOINT SUMMARY

- ◆ Mitosis is nuclear division in which the daughter nuclei have the same chromosome number as the parent nucleus, typically diploid nuclei producing diploid daughter nuclei
- ◆ The daughter nuclei are therefore genetically identical to the nucleus of the cell that produced them (except for any mutations that may occur)
- ◆ Mitotic cell division is seen in growth, repair and asexual reproduction.
- ◆ As a result of mitosis in the growth of an organism from the fertilised egg all cells therefore are genetically identical
- ◆ Similarly, offspring of asexual reproduction lack genetic variation
- ◆ The production of genetically identical cells retains the full set of genetic information needed for the replacement of any cells in repair processes, and in the asexual reproduction of organisms it allows for the rapid exploitation of optimum conditions where any variation would be disadvantageous
- ◆ The DNA replicates in interphase so that in the first stage of mitosis (prophase) the chromosomes appear as double structures of two chromatids (daughter chromosomes) joined at the centromere
- ◆ The nuclear spindle apparatus(NSA) forms from the dissolution of the nuclear membrane and the centrioles replicate and migrate to either pole of the NSA.
- ◆ The centromeres align on the equator of the NSA (metaphase)
- ◆ The centromeres divide and the chromatids are moved to the opposite poles (anaphase)
- ◆ Two new daughter nuclei are formed (telophase).
- ◆ Mitosis represents one phase of the cell cycle of division
- ◆ The cell cycle is completed by the formation of two new cells by division of the cytoplasm.

MEIOSIS

Meiosis is a special type of cell division called a **reduction division**. Meiosis occurs during the production of sex cells (gametes) in animals, and in spore formation which precedes gamete production in plants. Meiosis results in the number of chromosomes being halved from the normal **diploid** to the **haploid** number. The mechanisms of cell division are very similar to that already described for mitosis, but meiosis involves two divisions, referred to as the first and second divisions (meiosis I and meiosis II), and the result of meiosis is four cells (gametes or spores) each containing half the original number of chromosomes.

Meiosis involves certain genetic 'mixing' events, and these, along with the random fusion of the gametes in fertilisation to form a diploid zygote, result in genetic variation in the offspring which may be of adaptive advantage.



◆ CHECKPOINT SUMMARY

- ◆ Each gamete of a sexually reproducing organism contains one set of chromosomes (haploid)
- ◆ When two such gametes fuse in fertilisation they produce a zygote with two sets of chromosomes (diploid)
- ◆ Chromosomes in a diploid nucleus thus occur in pairs, each member of a pair carrying the same genes in the same relative positions (homologous chromosomes)
- ◆ Slight variations (alleles) of a gene at a particular locus can arise by mutation, so that an organism can either have an identical pair of genes at a locus (homozygous) or a pair that differs slightly (heterozygous)
- ◆ Pairs of sex determining chromosomes are only partially homologous e.g. the human Y chromosome has a short non-homologous arm which carries the male determining genes
- ◆ The production of haploid gametes from a diploid cell requires a reduction division (meiosis) in which the chromosome number is halved from diploid to haploid
- ◆ The production of haploid gametes ensures the maintenance of the diploid chromosome number in subsequent generations.

2.3

Genetic code

Contents

DNA as genetic material

- ▼ Evidence that DNA is the genetic material
- ▼ The structure of DNA, mRNA and tRNA in terms of nucleotides, base pairing and hydrogen bonding.

Nucleic acids and DNA replication

- ▼ The roles of nucleic acids in coding information, protein synthesis and the replication of DNA
- ▼ The semi-conservative replication of DNA

Protein synthesis

- ▼ The genetic code as a non-overlapping, degenerate code. Introns as non-coding DNA.
- ▼ The mechanism of protein synthesis involving the roles of mRNA, tRNA and the ribosomes.
- ▼ DNA determines the structure of enzymes which determine the phenotype of an organism

DNA as GENETIC MATERIAL

Evidence that DNA is the genetic material

DNA (deoxyribonucleic acid), is so named because it is an acid and it is found in the nucleus. It is a polymer made up of repeating sub-units, in this case, nucleotides linking up to form a polynucleotide. What makes DNA so important and so unique is that it contains within its structure the genetic code, and it can produce copies of itself (replicate).

The nucleus of cells contains mostly DNA and protein. For many years it was thought that protein was the hereditary material, but when it became possible to extract and measure the quantity of DNA, it was found that the DNA content doubled in cells just before dividing. It was also found that, in the formation of sex cells, the amount of DNA halved. Both pieces of evidence underline its central role in inheritance. Now it has become possible to analyse the molecular structure of DNA, to unravel the chemical code it contains, and to transfer pieces of this code between one organism and another in the process known as genetic engineering.

The structure of DNA and RNA

DNA is a polynucleotide made up of repeating nucleotide units. A **nucleotide** consists of three main parts: a pentose (5 carbon) sugar, joined to a phosphate group, and a nitrogen-containing organic base.

There are four different types of nucleotide in DNA each with a different organic base. The bases are **adenine** (A) and **guanine** (G) which belong to a group of chemicals called **purines**, and **cytosine** (C) and **thymine** (T) which belong to group called **pyrimidines**. Chains of nucleotides link up by condensation reactions, with the sugar and phosphate molecules forming the 'spine' of each strand of the molecule, with the bases to one side.

DNA consists of two such chains or strands linked by **hydrogen bonds** which form between pairs of bases. As the hydrogen bonds form, so the two strands of the DNA molecule twist into the spiral structure known as the double helix. Hydrogen bonds form only between specific pairs, namely A with T and C with G. The strands are therefore **complementary** to each other. The sequence along one strand determines the sequence of the other (complementary) strand. The understanding of the structure of the DNA double helix was one of the most significant breakthroughs in Biology.

RNA (Ribo(se)nucleic acid), like DNA, is a polymer made up of repeating nucleotides. It differs from DNA in the following three ways.

RNA is single stranded, not double stranded.

The pentose sugar in RNA is ribose, not deoxyribose (molecules of ribose contain an additional oxygen atom).

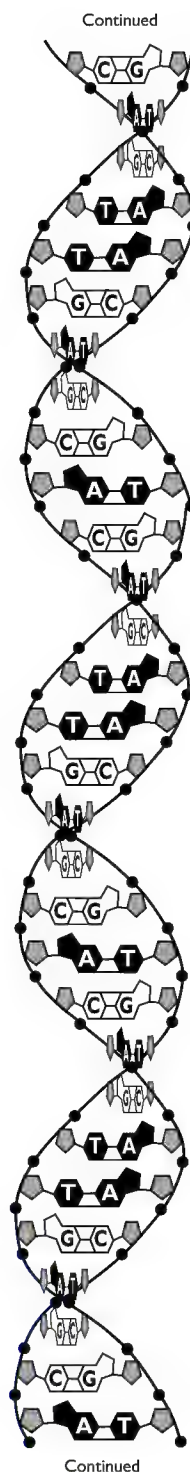
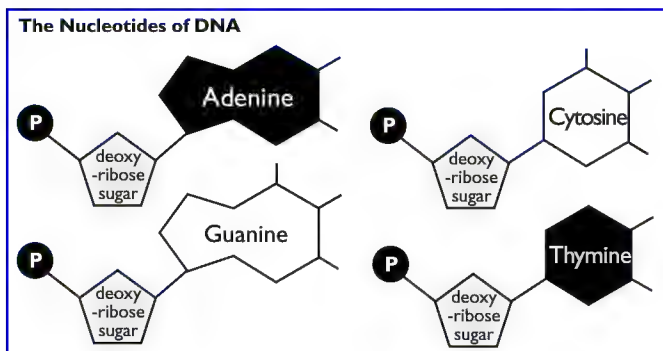
The pyrimidine base Uracil (U) is found instead of Thymine (T).

Living cells produce three different types of RNA in their nuclei, each designed to carry out a specific function in protein synthesis.

Messenger RNA (mRNA) consists of a single strand, between 75 and 3000 nucleotides long, produced in the nucleus as an exact copy of a selected section of the DNA code. The mRNA molecules travel out of the nucleus to the ribosomes where proteins are synthesised.

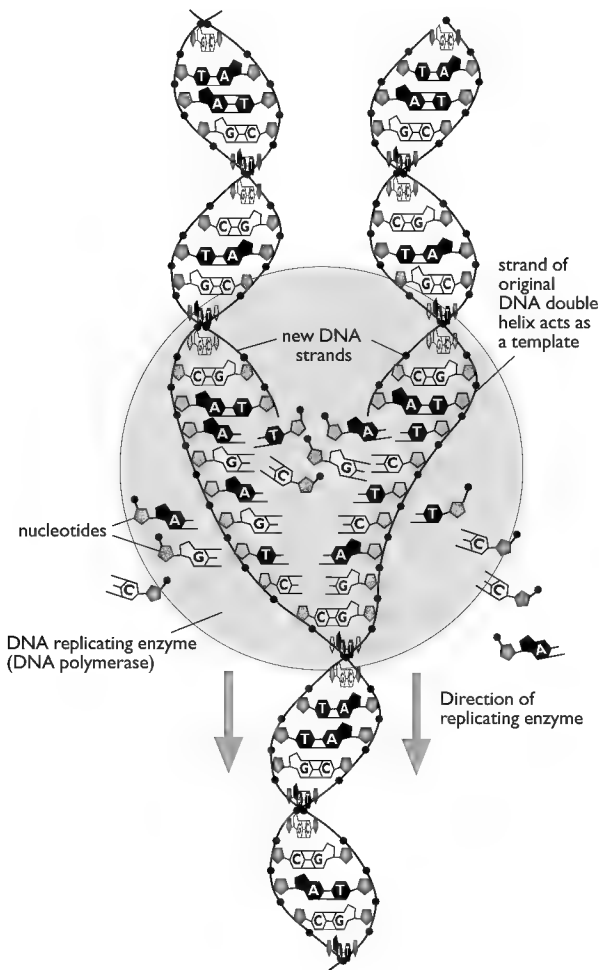
Transfer RNA (tRNA) is a shorter length of RNA, between 75 and 90 nucleotides long, twisted into a characteristic shape which allows one end to attach to a specific amino acid, and the other to expose a triplet of bases called the anti-codon which attaches to mRNA at the site of protein synthesis. tRNA molecules bring amino acids to their correct position as the proteins are being assembled. Each type of amino acid is carried by a different type of tRNA molecule with a specific anti-codon.

Ribosomal RNA (rRNA) consists of more than 1000 nucleotides and is found in ribosomes which are composed of an approximately equal mixture of proteins and rRNA. Ribosomal RNA is manufactured within the nucleolus.



Replication of DNA

In the process of DNA replication the original strands separate, and each acts as a pattern (template) for the formation of another new strand of DNA. Each of the daughter DNA molecules retains (conserves) one of the original strands and has one new one. For this reason, the process of replication is said to be **semi-conservative**. The process requires a supply of the four different nucleotides, energy in the form of ATP, and an enzyme called DNA polymerase, which is required to catalyse the condensation reactions by which free nucleotides form a copy of the template strand.



◆ CHECKPOINT SUMMARY

- ◆ The nucleus of cells contains mostly DNA and protein
- ◆ The DNA content doubles in cells just prior to dividing
- ◆ It was also found that, in the formation of gametes (sperms and eggs), the amount of DNA halved. This evidence underlines its central role in inheritance
- ◆ Now it has become possible to analyse the molecular structure of DNA, to unravel the chemical code it contains, and to transfer pieces of this code between one organism and another in the process known as genetic engineering
- ◆ DNA and RNA are long chain polynucleotide molecules with repeating sub-units of nucleotides
- ◆ Nucleotides consist of a 5 carbon sugar, a phosphate, and an organic base
- ◆ In DNA the sugar is deoxyribose and the organic base can be one of adenine, thymine, cytosine and guanine
- ◆ In RNA the sugar is ribose and the organic base can be one of adenine, uracil, cytosine and guanine
- ◆ The DNA molecule is a double stranded helix with the two strands held together by hydrogen bonds between complementary base pairs adenine-thymine and cytosine-guanine
- ◆ RNA is a single stranded helix with base pairs of adenine-uracil and cytosine-guanine
- ◆ There are three types of RNA - messenger, transfer and ribosomal
- ◆ DNA carries out semi-conservative replication in which each strand of the original molecule acts as a template for the construction of a new one, so that each new double stranded molecule of DNA consists of one original strand and a new complementary strand.

Experimental evidence for the semi-conservative mechanism of DNA replication includes experiments in which the mass of the DNA content of *E. coli*, grown on different nutrient media was determined and compared. One batch of microorganisms was cultured on a medium containing a 'heavy' isotope of nitrogen (^{15}N), the DNA was extracted and compared with that of organisms cultured on a medium containing normal 'light' nitrogen (^{14}N). The *E. coli* cells grown on 'heavy' nitrogen were then transferred to a new medium containing only light nitrogen and allowed to divide once to produce a new generation. The DNA of these daughter cells was found to be halfway between the 'heavy' and 'light' values, indicating that half the DNA was old and half was new (semi-conservative replication).

The genetic code

The DNA base sequence forms a four letter language (A,G,C,T) which, as you have seen, can be copied every time a cell divides. This DNA base sequence determines the sequence of amino acids in a polypeptide chain (the primary structure of proteins). A sequence of bases which codes for one polypeptide is known as a gene. Each gene codes for a different polypeptide chain. The primary structure of a polypeptide is the basis of the particular structural shape and characteristic of the final protein, e.g. enzymes, structural materials and membrane proteins. The base code determines the structure, functions and individuality of every organism.

Remember that up to twenty different types of amino acids may occur in a single protein. The DNA code has a four letter base language (A,G,C,T). A triplet of bases (sequence of three in a row) codes for each amino acid, for example 'CGC' codes for the amino acid alanine (Ala) whilst 'GCT' codes for arginine (Arg), so the code 'CGC-GCT-CGC' would be translated as 'Ala-Arg-Ala' in a

Amino acid	Abbr.	mRNA codons	Essential
Alanine	Ala	GCA, GCC, GCG, GCU	
Arginine	Arg	AGA, AGG, CGA, CGC, CGG, CGU	infants only
Asparagine	Asn	AAC, AAU	
Aspartic acid	Asp	GAC, GAU	
Cysteine	Cys	UGC, UGU	
Glutamic acid	Glu	GAA, GAG	
Glutamine	Gln	CAA, CAG	
Glycine	Gly	GGA, GGC, GGG, GGU	
Histidine	His	CAC, CAU	infants only
Isoleucine	Ile	AUA, AUC, AUU	E
Leucine	Leu	CUA, CUC, CUG, CUU, UUA, UUG	E
Lysine	Lys	AAA, AAG	E
Methionine	Met	AUG (Start codon)	E
Phenylalanine	Phe	UUC, UUU	E
Proline	Pro	CCA, CCC, CCG, CCU	
Serine	Ser	AGC, AGU, UCA, UCC, UCG, UCU	
Threonine	Thr	ACA, ACC, ACG, ACU	E
Tryptophan	Trp	UGG	E
Tyrosine	Tyr	UAC, UAU	
Valine	Val	GUA, GUC, GUG, GUU	E
STOP CODONS		UAA, UAG, UGA	

polypeptide. Note that the code is **non-overlapping**, i.e. it must be read 'CGC-GCT-CGC', not 'CGC-GCG-CGC-GCT-CTC-' etc.

Sixty four different triplet codes (codons) are possible with this four letter base language, more than sufficient to code for the twenty different types of amino acids. The term **degenerate** is applied to the genetic code because there are more codons available than can be used to code for amino acids. In fact most amino acids are coded for by more than one codon each. The process by which this code is used in protein synthesis, is described below.

Protein Synthesis

Protein synthesis involves all three of the RNA types described above working in combination, and is achieved in two stages. As the DNA cannot leave the nucleus, first the code has to be read and copied (transcribed) into mRNA, in a process called **transcription**. The mRNA moves out of the nucleus and associates with the ribosomes, where the code (now embedded in the mRNA) is used in the synthesis of a polypeptide in the process of translation.

Transcription begins as an enzyme, **RNA polymerase**, attaches to the '**Start**' codon of a gene. The DNA base sequence of this 'Start' codon is always 'TAC'. As RNA polymerase attaches to the DNA, the hydrogen bonds binding the two strands of the DNA double helix break apart to expose the sequence of triplet base codons on both strands. The enzyme then travels rapidly down the length of the gene like a zip fastener unzipping the DNA. As it does so, a molecule of mRNA is formed copying one of the DNA strands. Only one of the two DNA strands (the template strand) is copied into mRNA. The mRNA is therefore an exact replica (save that 'U' replaces 'T') of the other strand, consequently referred to as the **copy strand**. When the enzyme reaches the 'Stop' codon of the gene, transcription ends, the enzyme is free to start the process again, and mRNA is released out through a pore in the nuclear membrane to the ribosomes where the next stage occurs.

A complicating feature of the process of transcription in eukaryotic cells (cells with a nucleus) is that the length of DNA to be copied into mRNA (the gene) has sections of '**junk code**' which do not code for any of the required amino acids in a polypeptide chain. The 'junk' sequences are called **introns** and the parts to be transcribed and translated are called **exons**. The whole length is transcribed into mRNA, but before it is released from the nucleus, the introns are cut out with the assistance of enzymes, leaving a single, 'unpolluted', strand.

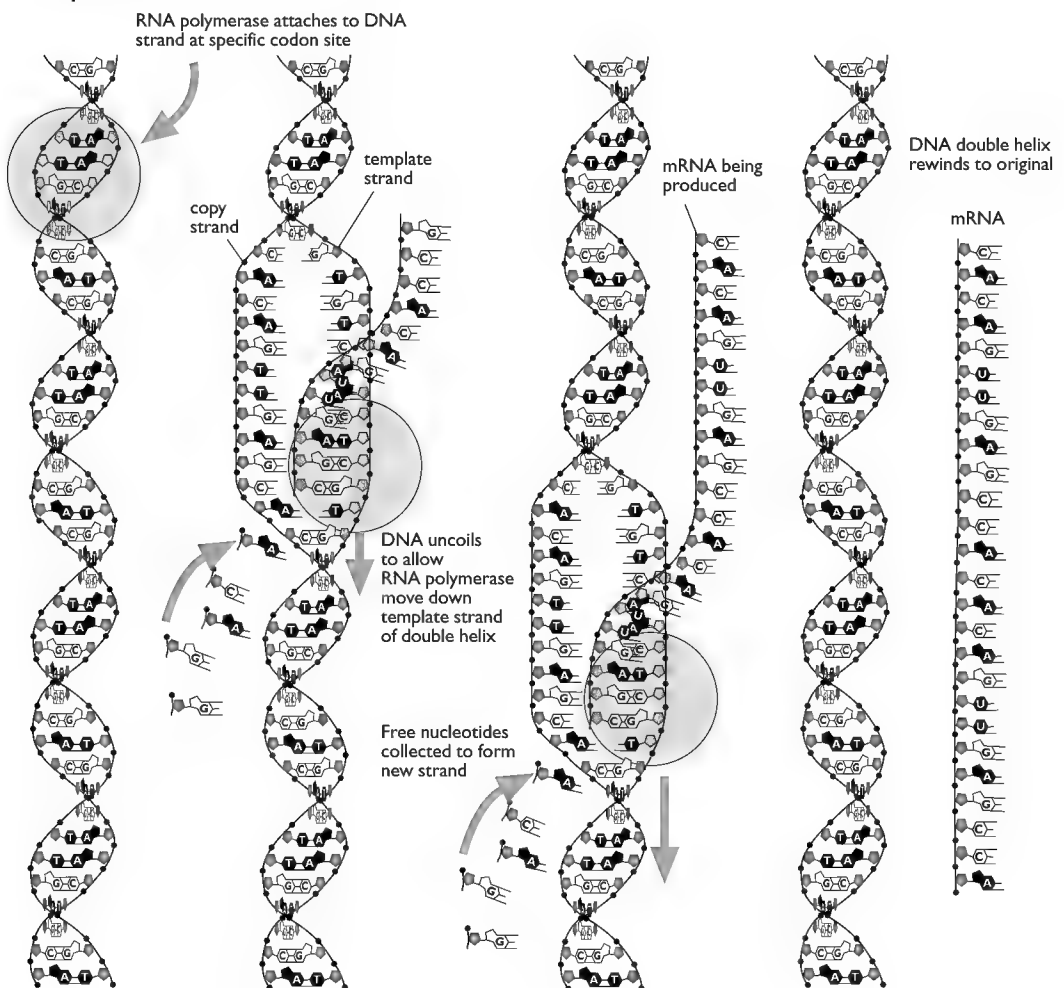
Translation occurs at ribosomes which are made up of two sub-units, one larger than the other, which come together only for the purpose of translating a length of mRNA. The mRNA binds to the small sub-unit at its 'Start' codon (AUG). It is then joined by the large sub-unit along with a molecule of tRNA which has the anti-codon 'UAC' and which carries the amino acid methionine. As the three different RNA types come together, the interface between the small and large sub-unit of the ribosome forms two binding sites allowing two triplet codons be lined up side by side. The process of translation can then proceed.

A second tRNA now occupies a position on the mRNA alongside the first and, as it does so, a peptide bond is formed between the two amino acids. The ribosome moves along the length of the mRNA, and

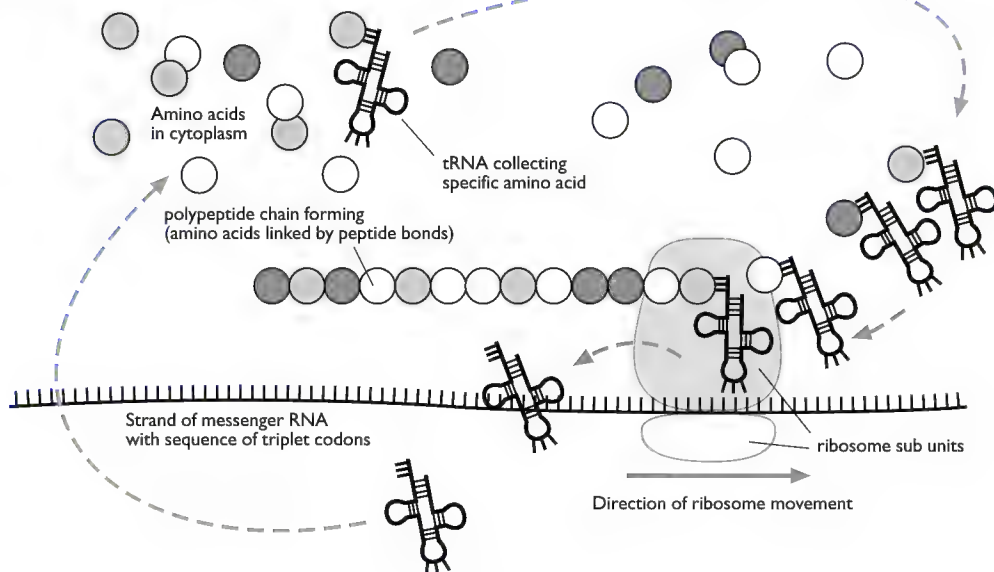
once a tRNA molecule has delivered the appropriate amino acid it is released to repeat the process. New tRNA molecules with their amino acids come to occupy the ribosomal coding sites, and the polypeptide chain grows ever longer. When the ribosome reaches the **'Stop' codon** (UAG) on the mRNA, it splits into its separate sub-units releasing the mRNA and the now complete polypeptide. A number of ribosomes move along the mRNA at the same time so that many polypeptide molecules are formed simultaneously. It is calculated that, in the average mammalian cell, more than a million new peptide bonds are being formed every second.

Generally mRNA molecules only have a short existence, sometimes just a few minutes, so that if continuous supplies of a particular protein is required the above processes must be repeated.

Transcription



Translation of mRNA code to build a polypeptide



◆ CHECKPOINT SUMMARY

- ◆ The sequence of bases (and hence nucleotides) on one strand of the DNA molecule carries the genetic code for the synthesis of proteins and hence ultimately living cells
- ◆ Three bases (a triplet codon) code for one amino acid
- ◆ There are 64 possible combinations of any three from four bases, but only 20 amino acids to be coded for
- ◆ Amino acids are coded for by more than one triplet codon and the code is said to be 'redundant' or 'degenerate'
- ◆ A gene is a sequence of nucleotides of a DNA molecule which codes for a polypeptide
- ◆ A gene for a polypeptide has a specific 'start' codon which thus sets the order of all the subsequent triplet codons
- ◆ Thus the code is non-overlapping i.e. a base can only be 'used' in one triplet codon
- ◆ Only a fraction of the nuclear DNA contains genes coding for proteins, the rest consists of so-called 'junk' DNA the function of which is not understood
- ◆ Nuclear DNA with the genetic code never leaves the nucleus in eukaryotic cells
- ◆ The genetic code for a polypeptide (a gene) is copied (transcribed) into mRNA which leaves the nucleus and associates with the ribosomes
- ◆ The ribosomes 'read' the genetic message on the mRNA and tRNA deliver amino acids in the sequence determined by the code.
- ◆ There are 20 different types of tRNA, each specific to a particular amino acid
- ◆ Each tRNA has a specific binding site at which the specific amino acid combines and an anti-codon which matches the specific codon for that particular amino acid
- ◆ The amino acids combine by means of peptide bonds to give a polypeptide chain
- ◆ The polypeptides subsequently form proteins
- ◆ As enzymes are proteins their synthesis is controlled by DNA.

DNA control of enzyme synthesis

Since the base sequence of DNA in a gene controls the production of one specific polypeptide or protein, it follows that any change in this base sequence (a gene mutation) may lead to the production of a faulty protein. Even a substitution or deletion of a single base can alter the translation of the DNA code into a sequence of amino acids in a polypeptide chain. If the primary structure of a polypeptide is altered this can affect its secondary, tertiary and quaternary structures, with corresponding effects on its function. The presence of such a faulty protein may in turn affect the appearance and functioning (phenotype) of the organism as a whole and cause a genetic disease or disorder. Gene mutations which occur during the production of gametes are thus the cause of genetic diseases in the offspring.

Some genetic diseases are referred to as 'inborn errors of metabolism' because they affect one or more of the metabolic pathways involved in the normal functioning of the organism. They may, for example, include diseases where particular enzymes necessary for metabolic functions are faulty or absent, e.g. Phenylketonuria (PKU). In this example the pathway from faulty gene to abnormal phenotype is clear. Identification of the gene and protein product responsible for the disease can allow medical intervention at any point in the pathway from gene to disease.

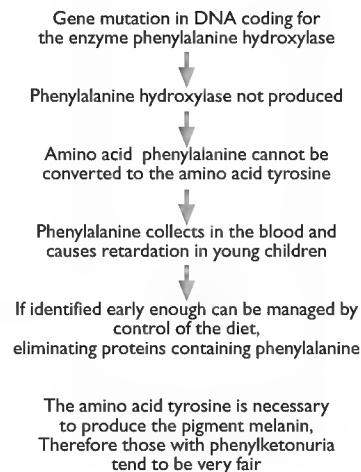
Phenylketonuria (PKU)

Most humans are able to convert some types of amino acids into other types of amino acid in the liver and thus produce all the proteins necessary for growth, repair and functioning of tissues. This explains why some amino acids are said to be essential (must be taken in the diet) whilst others are non-essential (do not need to be taken in the diet as they can be made from the essential amino acids in the body). Conversion of one amino acid to another (transamination) allows the body to make full use of ingested proteins and also prevents the build up of particular unused amino acids which could be dangerous.

In the disease phenylketonuria (PKU), however, a gene mutation in the DNA coding for the enzyme phenylalanine hydroxylase means that this enzyme is not produced in the liver. Without phenylalanine hydroxylase the body cannot convert the amino acid **phenylalanine** to the amino acid **tyrosine**. This results in an accumulation of phenylalanine in the body which severely affects many systems. Untreated sufferers may be both mentally and physically retarded.

PKU can be treated by modifying the diet to ensure that it lacks, or is very low in, the amino acid phenylalanine. The amino acid tyrosine may also be given. Under these circumstances individuals will not develop any signs of retardation. In the UK, all new-born babies are screened for PKU using a simple blood test which allows individuals with this genetic condition to be detected and treated before damage occurs. Treatment is only necessary in children, as adults seem to be unaffected by the high phenylalanine levels.

PKU sequence and effects



Phenylalanine amino acid
Collects in blood causing symptoms

Phenylalanine
hydroxylase
enzyme
ABSENT

Therefore no tyrosine formed

Therefore no melanin (pigment) formed

◆ SUMMARY OF PKU

- ◆ Gene mutation occurs in DNA coding for the enzyme phenylalanine hydroxylase
- ◆ Enzyme phenylalanine hydroxylase not produced
- ◆ Faulty metabolic pathway: amino acid phenylalanine not converted to tyrosine
- ◆ Phenylalanine accumulates with toxic effects
- ◆ Abnormal phenotype: Mental and physical retardation occurs.

2.4

Gene technology

Content:

Recombinant DNA

- ▼ The production of recombinant DNA and its use in the production of human insulin and other proteins
 - isolation of the required gene
 - use of the enzymes: reverse transcriptase, restriction endonuclease and ligase
 - sticky ends: insertion of the gene into a vector and its subsequent introduction into host cells; plasmids and viruses as examples of vectors
 - use of genetic markers such as genes conferring antibiotic resistance to detect genetically modified organisms
 - multiplication of host cells.
- ▼ The moral and ethical issues associated with recombinant DNA technology.

RECOMBINANT DNA

Imagine how simple life would be if there were only one type of computer and only one type of software. All the machines could then use the same language and all the information would be interchangeable. This is exactly how it is with the language of the genes. The synthesis of polypeptides from amino acids relies on exactly the same mRNA codons in every living organism so, for example, 'GCG' codes for the amino acid alanine and 'CGA' codes for arginine whether it occurs in a human, a honey bee or a mushroom. Now that genes can be identified and isolated, gene technology is providing a means for transferring them from one organism to another.

The terms 'genetic modification', 'genetic engineering' and 'gene technology' refer to the processes and techniques by which genes may be extracted from the DNA of one organism and inserted into the DNA of another organism (the **host** organism). In this way, the gene set (**genome**) of the host can be **transformed** (genetically modified). The modified DNA is called **recombinant DNA** because it has been re-combined to make a different set of codes.

Examples of genetically engineered products

Insulin is needed by people who are unable to produce sufficient quantities of the hormone and who would otherwise develop the disease **diabetes mellitus**. This is a condition, affecting 2% of the population, in which blood sugar rises to dangerous levels. Before gene technology, the only source of insulin was natural. It was extracted from the pancreases of pigs and cattle. This was not ideal because human insulin is slightly different in chemical structure, and although the pig and cattle version works on glucose in a similar way, some people are allergic to it and the process is both costly and open to contamination by impurities.

In the early 1980s the gene for human insulin was transferred from human pancreas cells to *E. coli* bacteria. Bacterial colonies modified in this way were cultured for mass production in industrial fermenters providing a constant supply of insulin. Now most insulin is produced by transformed bakers' yeast cultures. Yeast has various advantages over bacteria. It does not produce any toxic by-products, it can be grown on simple nutrient media, and it can secrete the finished product directly into the culture medium making the harvesting process easier.

Factor VIII is a vital blood clotting factor lacking in people suffering from **haemophilia**. Natural sources are both expensive and liable to contamination but now the gene for factor VIII has been engineered into yeast cells.

The food industry uses more and more genetically engineered products. One of the first to be developed on a commercial scale was the enzyme **chymosin** (rennilase) which is used to coagulate milk for the production of cheese. Chymosin occurs naturally in the stomach of young mammals, where it is often referred to as rennin. The cheese making industry relied on extracts from calves' stomachs until the advent of gene technology. Now chymosin can be obtained from modified yeast cultures grown in fermenters.

The techniques of Genetic Manipulation

Gene technology relies on the fact that the genetic language is universal; the 'software' (genes) can be used in any living organism. The difficulty lies in the 'cutting and pasting' of genetic information from one organism to another, and you must first become familiar with three enzymes which are crucial to this process.

Reverse transcriptase

Reverse transcriptase is an enzyme which makes a single strand of DNA from an RNA template. From this strand of DNA, a complementary strand can be assembled with the aid of DNA polymerase. Reverse transcriptase enzyme was identified and isolated from **retroviruses**, a group of viruses which includes the HIV virus. The genetic material of retroviruses is RNA (not DNA), and when retroviruses invade cells their RNA uses reverse transcriptase to make a piece of DNA. This becomes incorporated into the host cell's own DNA and directs the production of new viruses (an example of natural genetic modification of the host cell).

Reverse transcriptase is used in gene technology to make DNA from mRNA. Remember that mRNA is a copy of a single gene and that mRNA of a working gene may be found in the cytoplasm of a cell. It is possible to isolate a gene in the form of mRNA and then to multiply it using a combination of reverse transcriptase and DNA polymerase.

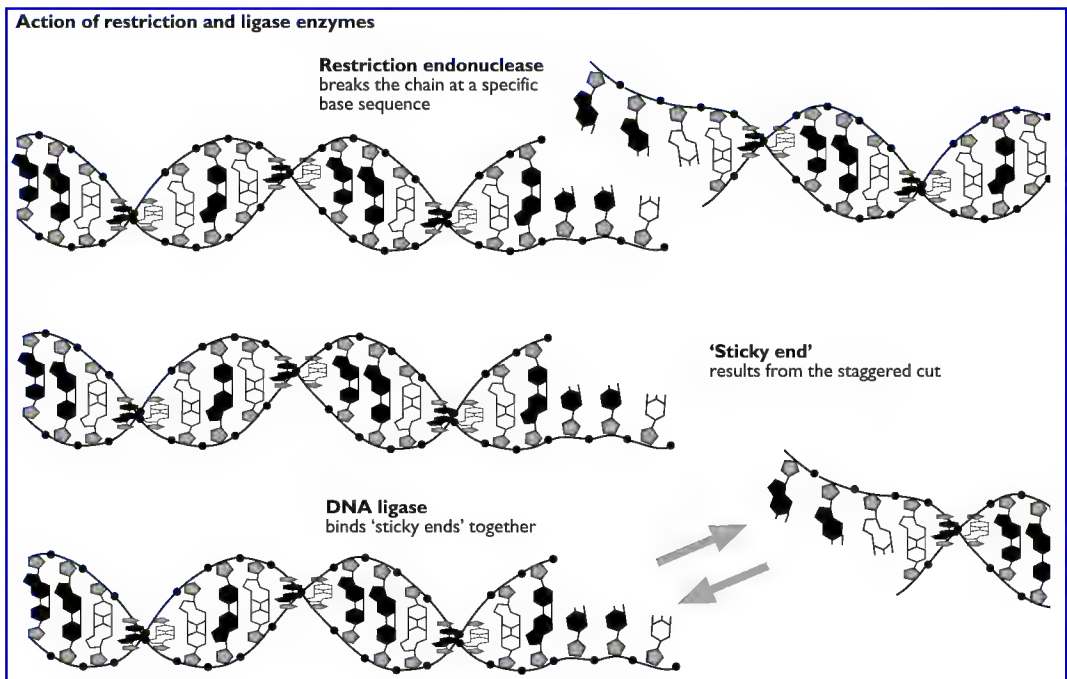
Restriction endonuclease enzymes

Another vital tool for gene technologists are enzymes called restriction endonucleases (restriction enzymes) which act like scissors to chop up DNA at specific base code sites into smaller polynucleotide fragments. Restriction enzymes are extracted from bacteria. In bacteria they serve to protect the organism from invading viral DNA. In gene technology they are used to cut DNA into fragments which can be separated and isolated. Often the cut leaves '**sticky ends**' which are exploited in another stage of the process.

DNA ligase

To 'ligate' is to join. DNA ligase is a joining enzyme. It is used to stick the sticky ends of DNA fragments together creating new combinations (**recombinant DNA**).

The techniques for transferring genes from one organism to another can be considered in five main stages.



Isolation of the required gene

It is surprisingly easy to extract DNA from living cells as you may already have discovered in the laboratory. Using restriction enzymes and gel electrophoresis it is possible to separate and isolate different DNA fragments, even pure genes. Alternatively, genes can be extracted from cells in the form of mRNA as described above and converted into DNA using reverse transcriptase.

The use of a vector

A vector is a carrier. The most common vectors in gene technology are bacterial plasmids and

bacteriophage viruses, both of which can carry the required gene to the host cell.

Plasmids are rings of DNA found in bacterial cells which are not part of the main bacterial chromosome. They are easily transferred from one bacterial cell to another. Plasmids can be cut at specific sites using restriction enzymes to leave sticky ends. If the same enzyme is used on the isolated gene then the sticky ends will match and the two can be joined using DNA ligase, so that the plasmid contains the recombinant DNA.

Bacteriophages are viruses which infect bacteria. They have a simple ring of DNA which they inject into the bacterial cell. This ring of DNA resembles a plasmid and can be modified in the same way to contain the required gene.

Transformation of the host cells

The vector is now brought into contact with the host cells, in the hope that some of the recombinant DNA might be taken up by the host cells. In order to discover whether or not the host cells have been transformed, **marker genes** (selection genes) are used. These are genes which are engineered into the vector alongside the required gene. They do not harm the host cell in any way but code for products which show up in an easily identifiable form in the host cell, confirming that the process of transformation is complete. One of the more common marker genes codes for antibiotic resistance. The transformed host cells can be isolated by introducing an antibiotic to the culture. All non-transformed cells are killed by the antibiotic, but the transformed cells survive as a result of the antibiotic resistant marker gene.

There has been some concern expressed about the use of antibiotic resistant genes. Opponents of this technique point to the risk of such genes escaping and entering disease organisms. Imagine the potential of a disease-causing bacterium, genetically modified to resist antibiotic treatment.

Multiplication of transformed cells

Once a cell or group of cells has taken up recombinant DNA, the next step is to culture the cells in a suitable growth medium, allowing them to grow and multiply. In the case of transformed bacterial and yeast cells, this can be done on a large scale in industrial fermenters, allowing the product(s) of the new gene to be harvested and purified, as in the case of insulin, cofactor VIII and chymosin.

Questions raised by Gene technology

Gene technology raises many hopes for the future. It should be possible for example to expand the tolerance range and extend the habitat of many crop plants by engineering resistance to salinity, heat, cold, and wind. New varieties will compete better with weeds and be more tolerant of attack from insects and fungal disease. Nitrogen fixation will be enhanced, and renewable energy crops will begin to make a realistic difference to the greenhouse gas levels. Malnutrition may be overcome by staple food crops with a complete complement of essential amino acids, and new drugs will be developed from plant substances.

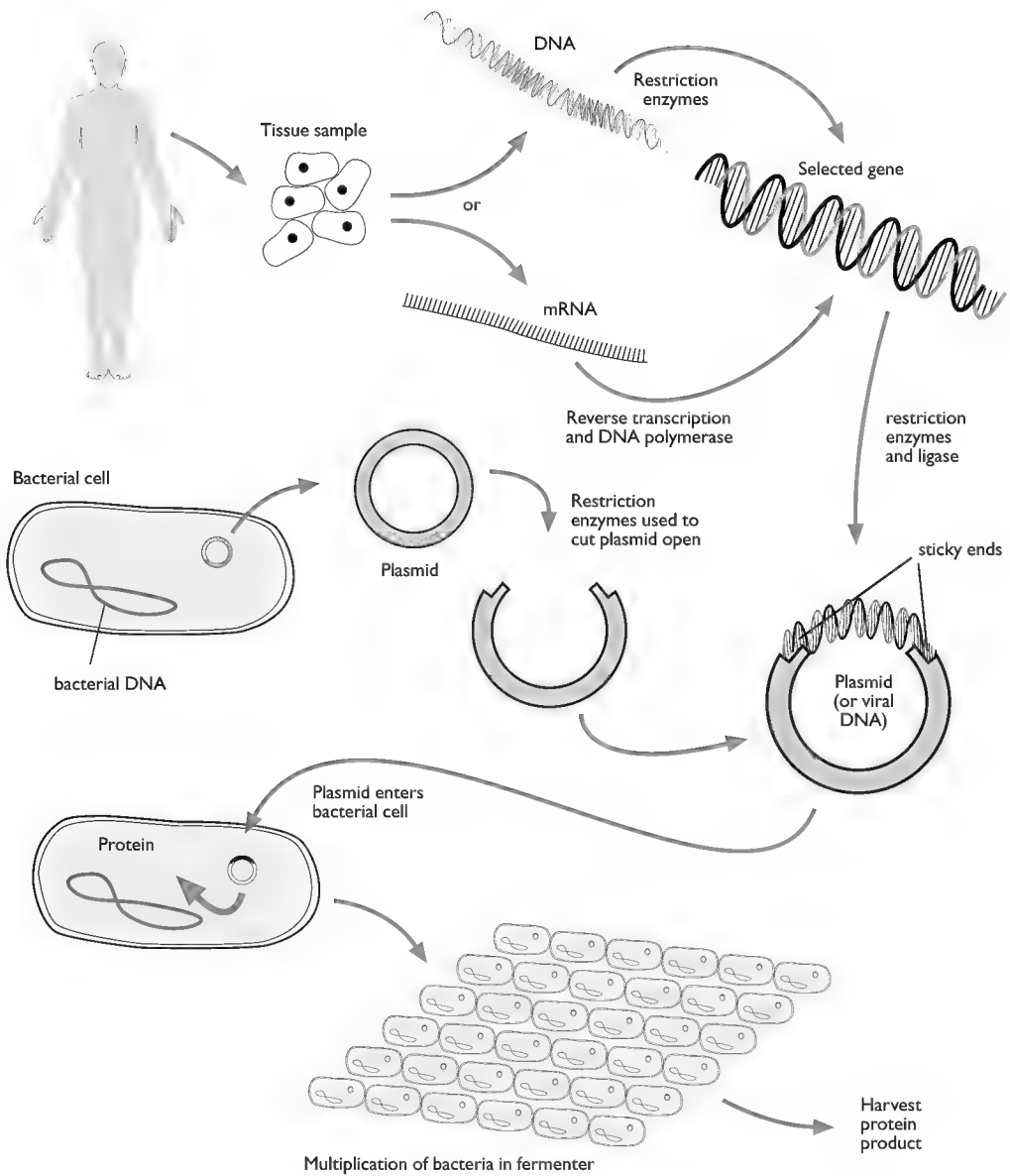
It also raises a number of questions about the way we want to live. Genetic modification has brought in the age of the designer plant, potatoes exclusively designed for oven chips, square tomatoes, straight bananas, salad vegetables, and fruits of every colour and texture. If there is a market for it, then the plant scientists will be able to engineer it. However, the accidental transfer of new genes into wild organisms could lead to herbicide resistant weeds and antibiotic resistant bacteria. Transgenic plants can also lead to transgenic pests and microbes.

The world population, currently about 5.5 billion, is expected to double to 11 billion by the year 2050. Given that 1 billion are already classified by international agencies as 'poor' and 500 million live in 'absolute poverty', it is clear that food production must be more than doubled in the next 50 years, and this must be achieved on a reducing area of fertile land.

Plant biotechnology, already moving at a rapid pace, offers some hope for the future. Plant biotechnology however, is largely the property of 'First World' independent companies, and 97% of the world's population increase will occur in the developing nations. Furthermore, environmental safety procedures, which evolve with the industry, exist in, and apply only to the countries which have the technology, yet transgenic products now have world wide distribution. A great deal of international cooperation, not to mention finance, will be needed to overcome the problem of safely transferring the products of the new technology to the countries which need them most. The human genome project which is analysing the sequence of bases in human DNA, also raises many problems to be dealt with. Certainly some inherited conditions and tendencies will be better understood, and various treatments developed. On the other hand the potential for abuse is enormous, including refusal of insurance cover to certain groups, employment vetting, immigration control and many others.

◆ CHECKPOINT SUMMARY

- ◆ Isolation of the gene to be manipulated is the first step
- ◆ It is easier to find and isolate the appropriate mRNA which has been transcribed (copied) from the relevant DNA, than it is to try and isolate the particular length of DNA itself
- ◆ The enzyme reverse transcriptase can then be used to make a copy of the DNA from the mRNA
- ◆ Plasmids (circular DNA) are isolated from bacteria
- ◆ Plasmids are cut open by restriction enzymes and the length of DNA is inserted and the plasmid resealed by ligase enzymes
- ◆ The genetically modified plasmids are then reintroduced into the bacteria
- ◆ The bacteria multiply and produce the protein coded for by the inserted DNA
- ◆ To identify those bacteria which have successfully taken up the modified plasmids marker genes are incorporated along with the required gene
- ◆ Marker genes include those for antibiotic resistance so that the culture can be flooded with antibiotic and only the bacteria with the marker gene will survive
- ◆ Two early successes with these techniques were the synthesis of human insulin for the treatment of diabetics, and blood clotting factor VIII for the treatment of haemophiliacs.



2.5

Forensic Examination of Blood

Contents

Principles of immunology

- ▼ Definition of antigen and antibody. The immunological response of B-lymphocytes
- ▼ ABO blood group antigens and agglutination

Genetic fingerprinting

- ▼ Genetic fingerprinting
 - restriction enzymes
 - electrophoresis
 - radioactive DNA probes

Polymerase chain reaction

- ▼ The polymerase chain reaction and its importance in obtaining increased amounts of DNA for analysis

PRINCIPLES OF IMMUNOLOGY

Definition of antigen and antibody

In order to remain healthy and able to function properly, the human body must protect itself from foreign material, particularly disease-causing organisms, known as **pathogens**. Initially it must aim to prevent pathogens from entering the body in the first place, and then it must be able to defend itself against them if they do enter. The first lines of defence are therefore the barriers to infection.

If pathogens do manage to pass through these barriers and enter the body tissues including the blood, an immune system is necessary to attack and destroy these pathogens and so prevent serious disease or death. There are two branches of the immune system in operation within the body which co-operate with each other to some extent.

◆ SUMMARY OF BARRIERS TO INFECTION

- ▼ impermeable skin which covers the body, unless broken, when the blood clotting process is rapidly activated to plug the wound and prevent pathogen entry
- ▼ the mucous membranes of nose and respiratory tract trap pathogens in sticky mucus
- ▼ anti-bacterial enzymes in saliva, sweat and tears
- ▼ stomach acid (pH 2).

These are:

- ▼ **Non-specific immunity** which is not targeted at specific types of pathogen but at foreign material in general. This involves phagocytosis and inflammation and is immediate.
- ▼ **Specific immunity** which uses B and T lymphocytes to target specific pathogens. Such responses are more complex and are often delayed. B-lymphocytes are concerned with **humoral immunity**, involving antibodies, whilst T- lymphocytes are concerned **cell-mediated immunity** with the direct killing of infected cells.

Immunology is the study of the mechanisms by which the B and T lymphocytes recognise and and destroy foreign material which enters the body. In all cases, foreign or 'non-self' material (in the form of pathogens, in the form of human cells from other individuals (as in blood transfusions or organ transplants, or, in the case of allergic reactions, in the form of molecules on common products like peanuts) is distinguished from 'self' material by the presence of 'non-self' antigens.

Antigens

Antigens are defined as molecules which, when foreign to an individual, stimulate an immune response by lymphocytes in the body. They are often protein or glycoprotein molecules found on the cell surface membrane of 'non-self' cells, but a range of other molecules also act as antigens. Antigens on the cell surface are important in cell recognition. Under normal circumstances the immune system of an individual will recognise all of their own cells as being 'self' owing to the familiar types of antigens present. Any cells (or other materials) with different 'non-self' antigens are rapidly detected by white blood cells known as B-lymphocytes and T-lymphocytes and targeted for destruction.

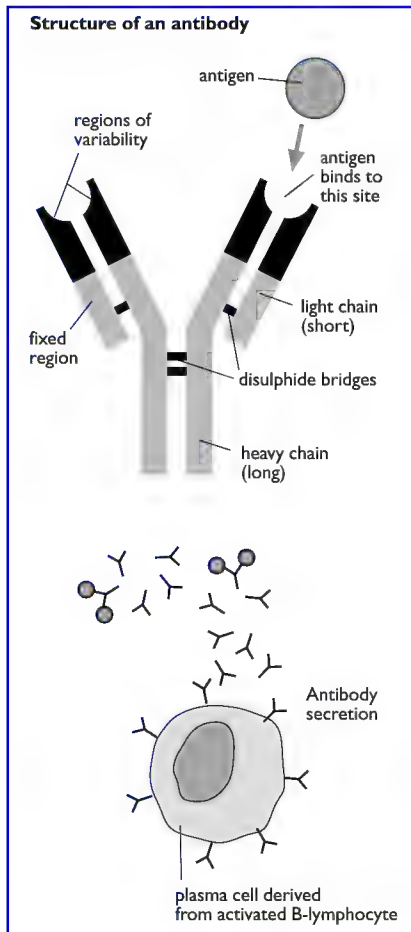
The immunological response of B lymphocytes

B-lymphocytes are involved in the production of antibodies in response to foreign antigens which is known as humoral immunity.

On the cell surface membrane of B-lymphocytes are a number of specific antigen receptors or membrane bound antibodies. These are sites which may become attached to antigens on the surface of pathogens or other foreign material, leading to a sequence of events in which free antibodies are produced to destroy the foreign material.

There are many thousands of specific types of B-lymphocyte and each is capable of recognising only one specific antigen. This allows specific antibodies to be produced in response to a vast range of antigens. For example, there is one type of B-lymphocyte which will attach to the bacterium causing TB and cause the release of antibodies against TB, and another type which attach to a red blood cell of blood group B in the body of a person with blood group A, and cause the release of antibodies against the group B antigens. This specificity is due to slight differences in the shape of the antigen receptor protein on each type of B-lymphocyte.

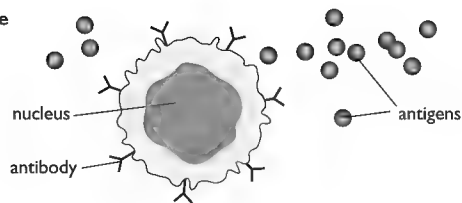
Antibodies are a type of protein molecule known as **immunoglobulins** which are secreted by activated B-lymphocytes when a specific antigen is encountered. They are shaped like a Y with the straight tail of the Y being known as the **constant region** since it is the same in every type of antibody. The forked part of the Y is known as the **variable region** contains antigen-binding sites which differ slightly in shape between each type of antibody. Antibodies do not directly destroy foreign material but target it for destruction by other branches of the immune system.



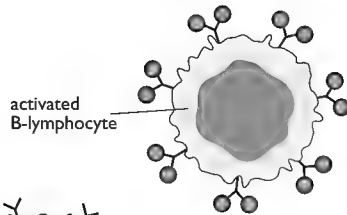
◆ SUMMARY OF THE IMMUNOLOGICAL RESPONSE OF B-LYMPHOCYTES

- ▼ Foreign material bearing antigens enters body
- ▼ Antigens on surface of foreign material come into contact with the specific antigen receptors on a B-lymphocyte
- ▼ Binding of antigen to antigen receptor activates the B-lymphocyte and causes it to divide by mitosis producing a clone of identical B-lymphocytes
- ▼ Most of the B-lymphocytes turn into plasma cells and the rest turn into memory cells
- ▼ The plasma cells begin to secrete specific antibodies against the foreign material concerned. Antibody molecules are secreted at a very high rate: up to 30,000 molecules per second
- ▼ The antibodies attach to the antigens on the foreign material and lead to the destruction of it in one of three ways:
 - By coating foreign cells or objects and causing them to stick together in such a way that they can then be engulfed by phagocytes (another type of white cell)
 - By binding to the foreign cells or objects at sites which interfere with their activities. For example, by covering the protein coat of a virus and preventing it from attaching to host cells.
 - By stimulating the release of other blood components called complement to lyse and destroy foreign cells
- ▼ Once the foreign material has been destroyed, the plasma cells eventually die and antibodies stop being secreted. But the memory cells remain in the lymph nodes and the circulation. If a subsequent infection with the same pathogen occurs then some of these will turn into plasma cells and start producing antibodies. This is the basis of natural active immunity to diseases and underlies the practice of vaccination (artificial active immunity).

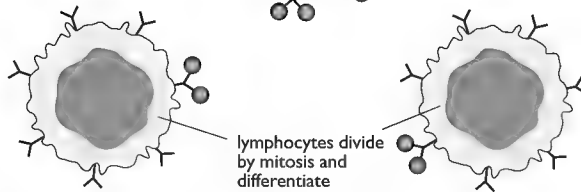
Immature B-lymphocyte



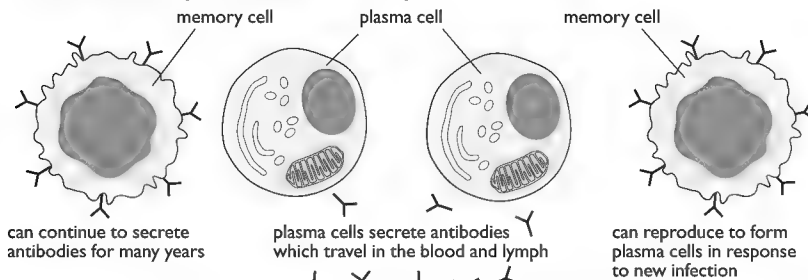
Activation



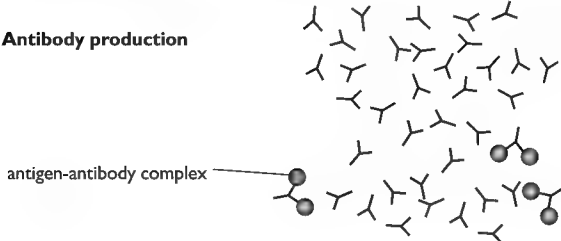
Division



Differentiation into plasma cells and memory cells

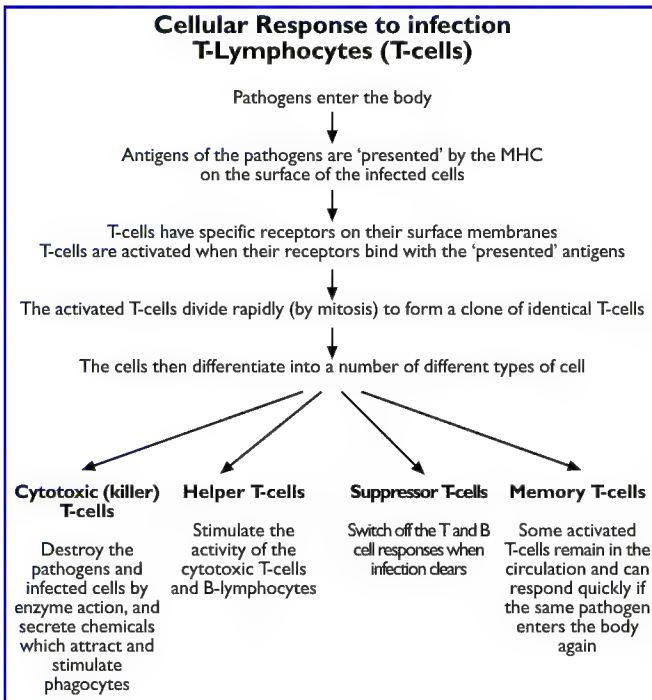


Antibody production



Immunological Responses of T-lymphocytes

The response of T-lymphocytes to foreign material differs from that of B-lymphocytes in that T-lymphocytes do not produce antibodies but work directly on infected cells. They may either lyse the cells directly by secreting enzymes or may interact with other branches of the immune system to effect their killing. As with B-lymphocytes, there are thousands of different types of T-lymphocyte each of which will only recognise one specific antigen.



ABO blood groups

In common with all human cells, red blood cells or erythrocytes have a number of proteins embedded into the cell membrane (see section 11.3), some of which are involved in cell recognition. As discussed above, such protein molecules are known as antigens or cell surface antigens and allow the immune system to distinguish between 'self' and 'non-self' cells. A number of different types of antigen occur on the red cell membrane, with the most well known being the ABO blood group system. Whilst the evolutionary significance of this genetic variation in humans is not well understood, its significance in medicine, and more specifically blood transfusion, is well established.

Group A individuals have A antigens on the surface of their red blood cells, Group B individuals have B antigens, Group AB individuals have both A and B antigens, and group O individuals have no antigens. The blood plasma of individuals of each of the four blood groups also differs in that it carries preformed antibodies against foreign ABO antigens. Thus a person of Group A will have antibodies against group B antigens (anti-B antibodies), and a person of Group B will possess anti-A antibodies. Since an AB individual has both A and B antigens on their red cells they will have neither anti-A or anti-B antibodies in their plasma. Group O individuals possess both anti-A and anti-B antibodies. As discussed above, if an antigen meets its corresponding antibody the antibody will bind to the antigen and may cause the foreign cells to clump together. Such a reaction, when blood of one ABO type is mixed with blood of another group, is known as agglutination.

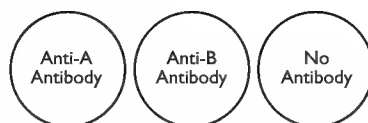
If blood of incompatible blood types are mixed in equal quantities agglutination will occur. In blood transfusions, however, generally a smaller volume of blood from the donor, is transfused into a larger volume of blood of the recipient, thus diluting the donor antibodies which are rendered relatively ineffective. Therefore in such transfusions it is only necessary for the **donor antigens** to be compatible with the **recipients antibodies**.

Blood group O is said to be the universal donor as their red cells have no antigens and can thus can be safely transfused into any individual without risk of antigen-antibody reactions. Group AB is the universal recipient, as since their plasma lacks anti-A and anti-B antibodies they can receive blood from any of the blood groups. Blood transfusions other than those indicated as 'safe' in the table below will lead to agglutination. This can cause rapid death of the patient as the agglutinated cells form blood clots which may block vital arteries within the body.

Recipient	Blood group			
Donor	A	B	AB	O
A	Safe	Agglutination	Safe	Agglutination
B	Agglutination	Safe	Safe	Agglutination
AB	Agglutination	Agglutination	Safe	Agglutination
O	Safe	Safe	Safe	Safe

Agglutination reactions noted above form the basis of blood group testing in hospitals and laboratories. Such rapid and simple tests are performed routinely in hospitals and many individuals are aware of their blood type even if they have never had a transfusion. Before an operation is begun the patient's blood type will be checked in case transfusions are needed.

Blood grouping

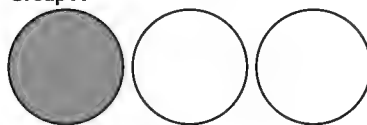


No Antibody Control

To check for contamination from area to area, and to eliminate appearance of blood clotting (which is different from agglutination) ie; if this area stays as unaltered blood the test is valid.

Drop of blood mixed in

Group A



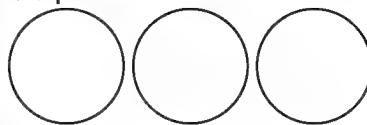
Group B



Group AB



Group O



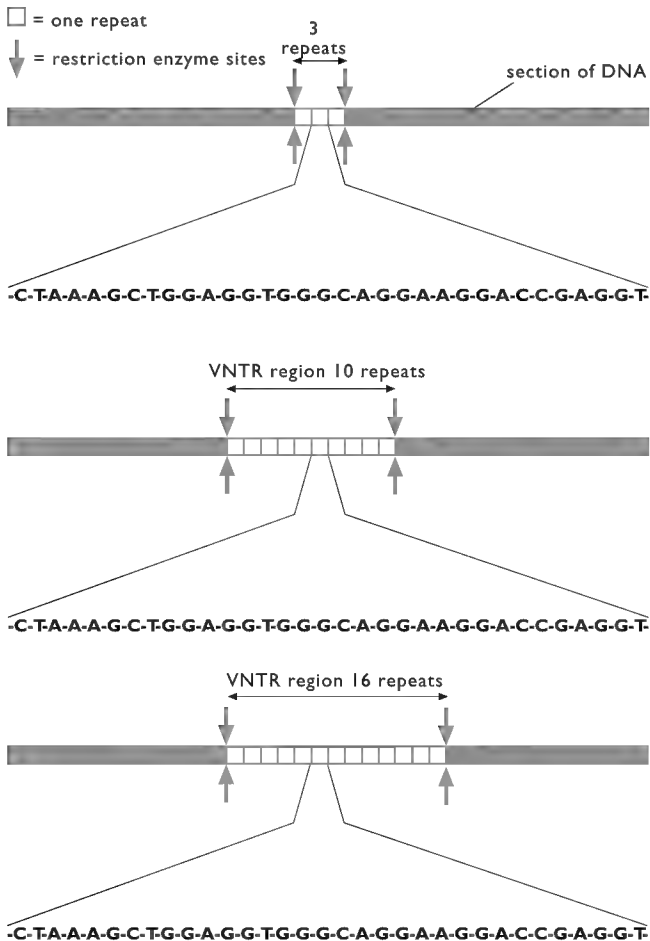
Invalid Test



GENETIC FINGERPRINTING

Genetic fingerprinting, also known as DNA profiling, is a recently developed technique which aims to reveal a specific and unique genetic pattern for each individual in the same way as each individual has a unique fingerprint. Its main application is in forensic science where, with reference to the DNA profiles of suspects or known criminals, it can help to identify the individual responsible for a crime from a small piece of DNA. It is also used in cases of disputed paternity or other family relationships. DNA profiles of organisms from other species also have applications in the fields of zoology and ecology where they can be used to assess genetic diversity and relatedness of animals (DNA profiling is expected to revolutionise the classification of organisms).

Instead of exploiting differences in genes, genetic fingerprinting relies on the extensive genetic variation which exists in the 'non-coding' DNA which lies between genes and even within genes (introns) and makes up 95% of the genetic content. Much of this non-coding DNA contains repeated sequences of sections of DNA between 15 and 100 bases long. Such regions are known as **VNTRs** (variable number of



tandem repeats) or **minisatellites**, and within them the number of repeats of a sequence is highly variable between individuals. Thus at a given site one individual may have a sequence of 17 bases (e.g GGGGGGAACAGCGACAC) repeated 75 times, whilst another may have 200 repeats and a third person 450 repeats. The DNA sequence immediately before and immediately after the sequence will, however be identical in all individuals.

Building up a DNA profile or genetic fingerprint of an individual requires analysing the DNA of that individual at several separate VNTR sites and gathering information on the numbers of repeat sequences. Some VNTRs exist as 'families' with the same repeated sequence found at many different locations throughout the genome: these can be analysed using 'multi-locus probes' described below. Other VNTR sites occur only once and require 'single locus probes'. The high variability of the sites between people makes the process very specific: as discussed later analysis of only a small number of VNTR regions can produce a unique profile.

Restriction enzymes

Restriction enzymes or restriction endonucleases are enzymes derived from bacteria which cut DNA at specific base sequences. Such enzymes will 'recognise' particular DNA sequences known as **recognition sites**, attach to these, and cut the DNA. The process of cutting the DNA is also called 'cleavage'.

In nature, the function of restriction enzymes is to cut out sections of viral DNA which have entered the DNA of the bacterium, but in genetic fingerprinting and other genetic techniques they are used to cut a length of DNA up into smaller pieces. There are several thousand types of restriction enzymes now in use, the names of which reflect the bacterial species from which they were extracted: thus EcoRI is derived from *E. Coli* and HindII from *Haemophilis influenzae*. Each restriction enzyme is specific to one base sequence and will digest or 'cleave' the nucleic acid (DNA) whenever such a site is encountered but at no other point. It should be noted that some cut straight through both strands of DNA creating 'blunt ends', whilst others produce a staggered cut or 'sticky end' which is useful in genetic engineering.

To create a DNA fingerprint of an individual the first stage is to mix a sample of DNA with restriction enzymes which cut at the two ends of one or more known VNTR sites. (Remember that such sites will have a common start and end sequence even though there are variable numbers of repeats in between.) The fragments produced will vary in length depending on the number of times the DNA sequence is repeated. It is important that there are no restriction enzyme sites within the repeat sequences as this would prevent analysis of VNTR regions on the basis of size.

Electrophoresis

This is a technique used in the analysis of DNA which has been cut into fragments of differing sizes by restriction enzymes. It is based on the principle that smaller fragments of DNA will migrate through a gel faster than larger ones when a direct electric current is applied across the gel (which is in a buffer solution). The reason for this is that the larger fragments have more difficulty moving through the

SUMMARY OF GENETIC FINGERPRINTING

- ◆ Creating a genetic fingerprint from the DNA of an individual involves a number of stages:
- ◆ Extraction of the DNA from cells (may be white cells, sperm cells in semen, hair cells etc)
- ◆ Amplification of the DNA using the polymerase chain reaction (PCR)
- ◆ Use of restriction enzymes to cut the DNA at specific sites either side of VNTR region
- ◆ Use of gel electrophoresis to split the DNA fragments up on the basis of their size (i.e on the number of repeats)
- ◆ Southern blotting and use of radioactive DNA probe to locate the fragments of DNA
- ◆ Autoradiography to create an image of the DNA pattern for interpretation.

Enzyme	Source Organism	Restriction Site
EcoRI	<i>Escherichia coli</i>	↓ -G-A-A-T-T-C- -C-T-T-A-A-G- ↑
EcoRII	<i>E. coli</i>	↓ -G-C-C-T-G-G-C- -C-G-G-A-C-C-G- ↑
Hae III	<i>H. aegyptius</i>	↓ -G-G-C-C- -C-C-G-G- ↑
HpaII	<i>H. parainfluenzae</i>	↓ -C-C-G-G- -G-G-C-C- ↑
SmaI	<i>Serratia marcescens</i>	↓ -C-C-C-G-G-G- -G-G-G-C-C-C- ↑

dense gel. All fragments will however move, as they are negatively charged and move towards the positive terminal.

To perform electrophoresis, a gel is made from agarose (a polysaccharide) and placed in a tank of buffer solution. DNA samples that have been treated with restriction enzymes are loaded into wells in the gel along with a dye solution. This ensures that all have equal electrical charge and that their position can be marked. A direct current is passed through the buffer solution causing the DNA marked by the dye to move through the gel. Once the dye front has migrated about three quarters of the way down the gel the current is switched off. The position of DNA fragments on the gel cannot be seen at this stage: further steps are required for this.

Southern Blotting

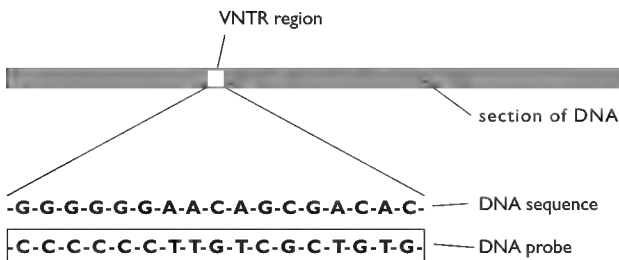
The first stage in allowing visualisation of the DNA fragments is known as Southern Blotting. There are two basic stages involved:

- ▼ firstly the DNA on the gel needs to be heated to cause the two strands of the helix to unwind making single stranded DNA;
- ▼ secondly this single stranded DNA is transferred from the gel to a nitrocellulose filter or a nylon membrane. The membrane will bear an exact copy of the gel in terms of the positions of DNA fragments. It is very important to make the DNA single stranded: if this is not done then the gene probe described below cannot bind.

DNA probes

A DNA or gene probe is a single stranded DNA sequence usually 10 nucleotides or more in length which is complementary to a stretch of known DNA (e.g. part of a repeat sequence in a VNTR region) on a chromosome. The probe is radioactively labelled using ^{32}P (this radioactive phosphorus is incorporated into the nucleotides which make up the probe). The probe is added to the nitrocellulose filter (produced via Southern blotting) where it will attach to single stranded DNA of a complementary sequence. This is known as hybridisation.

DNA in a VNTR and DNA probe



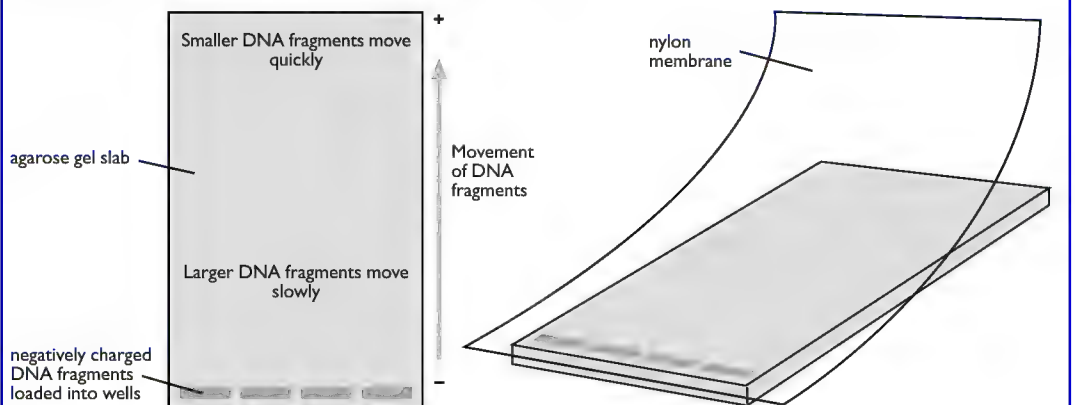
The probe will bind to any region with this sequence regardless of the number of repeats that exist. As discussed above, copies of a multi-locus probe will bind to more than one site as it has a complementary sequence that is common to several VNTR sites. A single locus probe will detect and bind to only one VNTR site in the entire DNA of an individual.

Autoradiography

In order to visualise the bound DNA probe and hence detect the size of DNA fragments separated in the original gel an X-ray film is placed over the nitrocellulose filter. At sites where the gene probe has bound to DNA fragments the radioactive P will create a black band on the film.

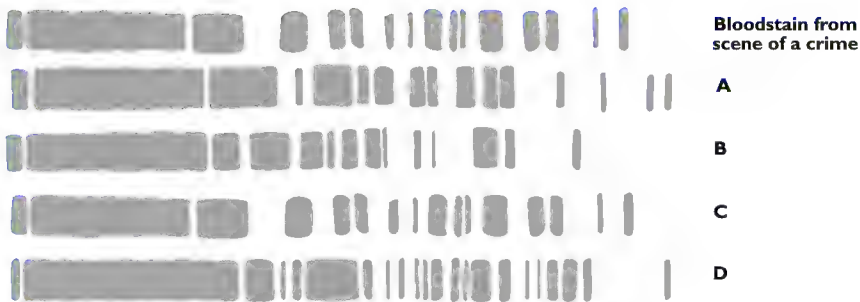
Analysis works as follows. A black band at the bottom end of the picture will correspond to small DNA fragments to which the probe has bound; i.e.. a VNTR site with a small number of repeats which has migrated a long way through the gel. Conversely black bands at the top of the picture reveal large fragments to which the probe has bound. The actual lengths of the fragments and hence the number of repeats can be compared to known lengths in marker DNA loaded into the gel. Each individual will have two bands for each VNTR which may be the same or different. Lengths of DNA are measured in kilo bases (Kb) (1 Kb = 1000 bases).

- 1 Extracted DNA e.g. from white blood cells, semen etc.
- 2 DNA cut into pieces by **Restriction Enzymes**
- 3 DNA pieces are separated by **Gel Electrophoresis** this takes 24 hours

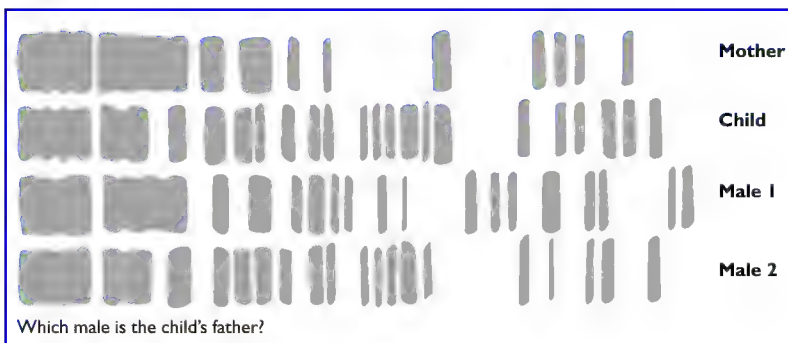


- 4 **Southern Blotting**, the DNA fragments are transferred (blotted) onto a nylon membrane laid on top of the gel slab.
- 5 **Gene Probe Treatment** - the nylon membrane is put into a tank of single strand DNA's (probes) which are complementary to specific core DNA sequences and bind to them. The probes may be radioactive or fluorescent. Excess probes are washed off
- 6 X Ray film is placed on the membrane and the typical bands appear where the presence of **radioactive/fluorescent probes** fog the film.
- 7 The X Ray film is processed to form the **DNA Fingerprint** with its characteristic 'barcode' pattern of dark bands.

Genetic Fingerprinting - Autoradiograph bands



Was individual A,B,C or D more likely to have been present at the scene of the crime?



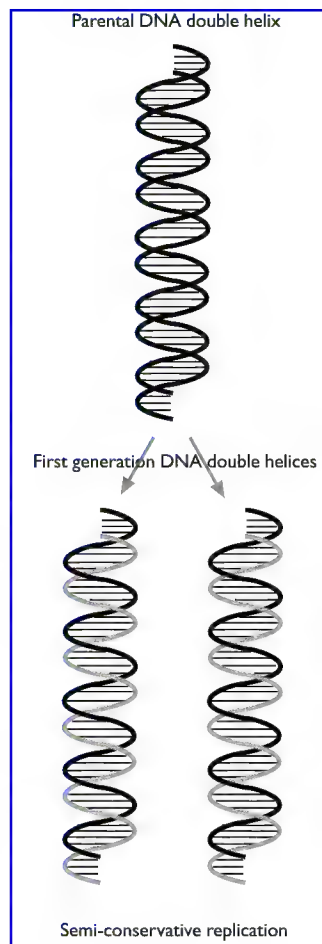
Reliability of genetic fingerprinting

As discussed above, genetic fingerprinting is used in forensic investigations where DNA collected at the scene of a crime can be analysed and compared to the genetic fingerprints of known individuals, or to fingerprints prepared from unknown individuals. For reliable comparisons several VNTR sites must be compared. It is estimated that if one single locus probe is used the same pattern of bands will occur in 1 person out of 100. But when 2 are used the number jumps to 1 in 10,000. With several probes the chances of two sets of DNA matching are so low that the test becomes highly specific in identifying individuals.

It should be noted, however, that whilst genetic fingerprinting can theoretically identify a single individual from the DNA contained in one cell it cannot stand alone in forensic or other investigations, but only as a back up to other types of evidence. For example, finding a human hair at the scene of a crime may allow you to make a genetic fingerprint of the person from whom it came. With reference to a genetic database this could allow an individual to be identified. But without other evidence this cannot be used to convict a person: the hair could have come from someone visiting the area prior to or after the crime, been 'planted' on purpose, or could have blown in. Furthermore, the genetic fingerprint obtained from DNA found at the scene of a crime may not be clear, the DNA may have partly decomposed or the sample could be contaminated.

POLYMERASE CHAIN REACTION (PCR)

PCR is a technique used to amplify (make many copies of) a DNA molecule or part of a DNA molecule. Based on an understanding of the semi-conservative mode of DNA replication in nature, PCR has revolutionised genetic technology. It theoretically allows any number of copies of DNA to be produced from a single DNA molecule (the template). Often it is used to amplify a known section of DNA only. PCR is crucial in generating copies of DNA for use in such processes as: genetic fingerprinting and genetic screening for diseases.



Amplifying a piece of DNA by PCR requires the 4 'ingredients' listed below

- ▼ DNA. Theoretically a single DNA molecule is sufficient, but usually a larger amount of DNA is used.
- ▼ Primers. These are short single stranded pieces of DNA which bind to the parent DNA strands and initiate the synthesis of a complete new strand of DNA on the template of the parent strand. One primer is needed for each parent DNA strand.
- ▼ A supply of each of the 4 nucleotides containing the bases A, C, T and G. The bases on the free nucleotides will pair with their complementary bases on the DNA parent strand (A with T and C with G) thus synthesising the new strand.
- ▼ The enzyme Taq polymerase. This enzyme works like human DNA polymerase, catalysing the attachment of the complementary bases to one another. Taq polymerase is, however, thermostable (temperature resistant) as it is derived from the bacterium, *Thermus aquaticus*, which inhabits hot springs. This is important, as PCR requires high temperatures to work.

The reaction mixture made of these 4 ingredients is placed in an individual well on a plastic plate, or in individual tubes with a layer of oil on top to prevent evaporation. The reactions involve alternate heating and cooling of this mixture and are performed by a PCR machine. The machine can be programmed for different numbers of cycles, the more cycles the more copies of the DNA are produced.

Polymerase Chain Reaction (PCR)

Allows short lengths of DNA to be copied millions of times.

Process is very rapid - each cycle takes about 5 minutes.

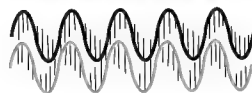
Four ingredients required:

- A** length of DNA helix to be copied;
- B** four types of free nucleotides;
- C** Primers - short, single stranded pieces of DNA (about 20 bases long) complementary to bases at the start of the portion of DNA to be copied;
- D** DNA polymerase enzyme.

1 Original double strand DNA

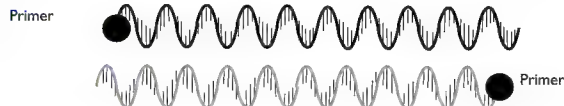


2 High temp. (96°C) strands uncoil

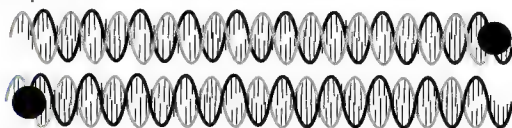


3 DNA primers DNA polymerase and nucleotides added

4 Temp reduced DNA 'Primers' attach (anneal) to the strands to be copied



5 DNA (Taq) polymerase organises free nucleotides to form complementary strands DNA is replicated



6 Process repeated many times very rapidly The original length of DNA has been amplified. Used to copy DNA from bones, blood, mummies, insects in amber, etc.

◆ SUMMARY OF THE STAGES OF PCR

For simplicity the stages of PCR are described here as if a single complete DNA molecule is used as the starting point

- ◆ PCR mixture is heated causing the DNA double helix to unwind exposing the bases
- ◆ The mixture is cooled and primers bind to the ends of each DNA strand. This is known as annealing
- ◆ Taq polymerase allows free nucleotides to pair with the exposed bases on both DNA strands. A pairs with T and C with G. Binding begins immediately after the primer sequence and continues until the whole complementary strand has been synthesised. Two new DNA double helices result. Each contains one of the original parent strands. One complete cycle has now been completed
- ◆ The process is repeated starting with two copies of the DNA and ending with four: (Each DNA molecule unwinds, has a complementary strand synthesised and winds back up). As the process is repeated over and over again the number of copies of the DNA increases geometrically: 2 → 4 → 8 → 16 → 32 → 64 → 128 etc.
- ◆ The DNA produced is used in genetic fingerprinting, genetic screening or other genetic studies. Alternatively it may be frozen and stored for future analysis.

2.6

Cultivated plants and the manipulation of the environment to increase productivity

Contents

Adaptations of cereals

- ▼ Structural and physiological adaptations to different parts of the world
 - Rice as a swamp plant with hollow aerenchyma and a tolerance to ethanol produced by anaerobic respiration.
 - Sorghum as a plant which grows in hot, dry conditions; its xerophytic modifications include the presence of an extensive root system, a thick cuticle and a reduced number of sunken stomata; both the adult plants and the embryos can tolerate high temperatures.
 - Maize as a tropical plant with a specialised method of photosynthesis; the advantages of this method of photosynthesis in increased efficiency at high temperature and low carbon dioxide concentrations.

Controlling the abiotic environment

- ▼ Human control of the abiotic environment of crop plants
- ▼ The effect of light intensity, temperature and carbon dioxide concentration on rate of photosynthesis and productivity. Enhancement of these factors in commercial glasshouses.

Fertilisers

- ▼ The use of fertilisers to replace nutrients lost by harvesting crops.
- ▼ The advantages and disadvantages of organic and inorganic fertilisers. The relationship between yield and quantity of fertiliser added. The environmental issues arising from the use of fertilisers. Leaching and eutrophication.

Pesticides

- ▼ Interspecific competition between weeds and crop plants. Reduction of crop yield by insects either directly or indirectly by reducing the photosynthetic tissues of the plant.
- ▼ The principles of using chemical pesticides, biological agents and integrated systems in controlling pests of agricultural crops.
- ▼ The environmental issues associated with pest control. Toxicity and bioaccumulation.
- ▼ Evaluation of the issues involved in using different methods to control pests

ADAPTATIONS OF CEREALS

Cereals are seed crops derived from the family of plants known as the Graminae, or grasses. They have formed the main energy providing component (staple food) of most human diets since our early ancestors turned from a hunter-gatherer existence to the collection and cultivation of selected wild grass seeds. The civilisations of the ancient world were founded on settled agricultural communities and their cereal crops; rice in the Far East, wheat and barley in the Middle East, *Sorghum* in Africa, and maize in Central and South America. All of these crops could be eaten as whole grains, ground into flour and baked into bread of some kind, or simply mixed into a porridge-like paste.

Rice

The ancestral grass plants from which rice has been developed were adapted to live partially submerged in marshy regions. It is possible to grow rice on dry land like wheat, even in semi-temperate areas but the yield increases up to three times when it is grown in paddy fields in tropical or sub-tropical temperatures. Over half the world production is in irrigated soil, notably in the great deltas of Asian rivers such as the Ganges, Irrawaddy and Mekong. Modern varieties are fast growing, taking as little as 120 days from seed to harvest, and it is common for three crops to be produced each year. For most of the growing period the rice plants are partially submerged but the land is drained just before harvest so that the dry seed can be collected efficiently.

Most plants can not survive flooding. This is because plant roots require a good supply of oxygen for aerobic respiration. Remember that the uptake of minerals is an active process requiring high energy levels. In the absence of oxygen the roots will respire anaerobically but this can not be tolerated for long because they are soon poisoned by the ethanol released as a bi-product. Rice roots have an unusually high tolerance to ethanol and their seeds can germinate in oxygen starved soils. In common with other water adapted plants they also develop an interconnecting system of large air spaces linking the roots with the leaves and other parts above water. This specialised tissue is called **aerenchyma** and it allows the flooded roots to breathe.

Rice seeds are stripped of their outer coat and embryo (the parts containing proteins and minerals) by a process called 'polishing' to produce the product which you find in the supermarkets. It is virtually pure starch and provides virtually all of the daily energy intake for over 50% of the world's population.



Maize

The ancestry of maize is uncertain. There are no modern grasses which are obviously related to it, but it is certain that the plant originated in Central America, probably in Mexico. It thrives in hot climates with well watered soils, and is adapted to photosynthesise successfully at light intensities and temperatures higher than most plants can tolerate, and with a reduced supply of carbon dioxide.

When all other conditions for photosynthesis are at their optimum level, the rate of carbohydrate production will increase with increasing light intensity until it reaches a maximum value called the **light saturation point**. At high levels of illumination the enzyme Rubisco, which normally catalyses the fixation of carbon dioxide into sugars, starts to react with oxygen in a process called **photorespiration**. Photorespiration uses up oxygen and releases carbon dioxide. It yields no useful energy and is therefore an essentially wasteful process consuming up to 25% of the carbohydrate manufactured by photosynthesis. In order to recapture the lost carbon dioxide, plants are required to open their stomata at the hottest and brightest times of day, thereby suffering a further loss of water by transpiration. This is particularly significant in tropical climates where light intensities commonly exceed the light saturation point. Maize and some other tropical plants, notably sugar cane and *Sorghum*, have evolved a mechanism referred to as **C4 photosynthesis** which overcomes the problem of photorespiration by increasing the concentration of stored carbon dioxide in their leaves. This gives them a higher light saturation point, and therefore a higher productivity.

Maize is most commonly encountered in the form of sweet corn, pop corn or tortilla chips in Western Europe. In Central and South America, maize flour is the basic material for the manufacture of tortillas, tamales and a variety of drinks. It is the single most important crop in the United States although 90% of the production is for animal feed.



Maize crop



Male flower



Female flower

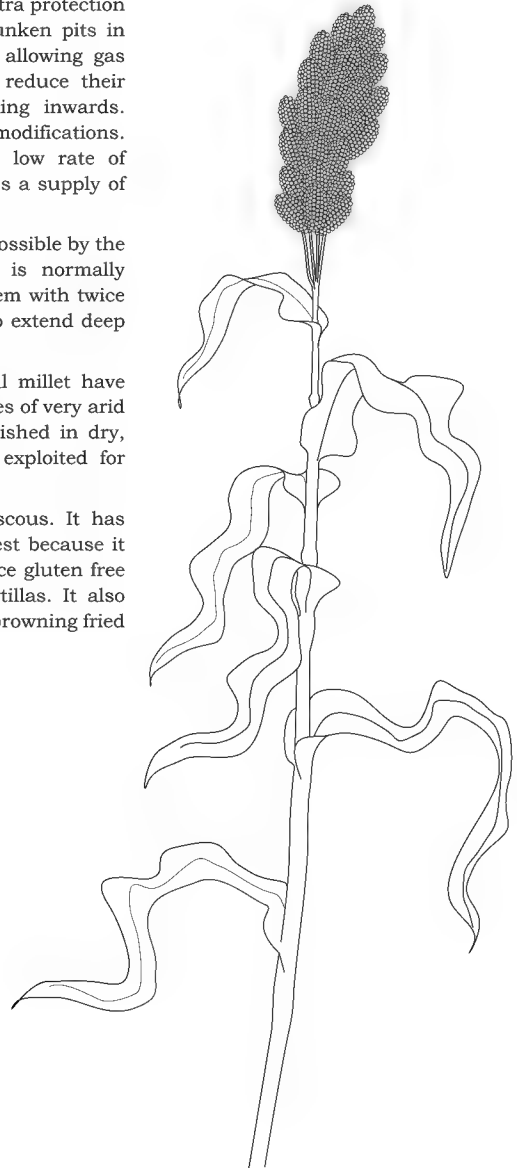
Sorghum

Like maize, sorghum is a C₄ plant, specialised to maximise photosynthetic yield in high light intensities and hot climates. Compared to maize, however, *Sorghum* is able to tolerate dry conditions, requiring 20% less water to produce equivalent yields of dry matter, and is able to grow in more hostile climates than any other cereal crop. Plants which are specially adapted to tolerate dry conditions are called **xerophytes**. The adaptations (xerophytic features) centre on ways of reducing water loss whilst retaining photosynthetic activity. Sorghum is similar to maize in its general structure but its leaves are more specialised for water conservation. They are coated with a white waxy layer, giving them extra protection from desiccation, and the stomata are situated in sunken pits in order to reduce unnecessary water loss, whilst still allowing gas exchange. In conditions of water stress, the leaves reduce their exposed surface area by becoming erect and curling inwards. Photosynthetic production is not hampered by these modifications. Sorghum retains high photosynthetic yields with a low rate of transpiration. As in maize, the C₄ mechanism provides a supply of carbon dioxide even when the stomata are closed.

Survival of grasses over extended dry periods is made possible by the possession of a much larger rooting system than is normally necessary. Sorghum has a very extensive rooting system with twice as many side branches as that of maize. Its roots also extend deep enough to extract water in drought conditions.

Sorghum and the even more drought resistant cereal millet have become the staple foods of people living on desert fringes of very arid land. These crops have also been successfully established in dry, nutrient depleted and wind eroded soils previously exploited for cotton production.

Sorghum is used to make bread, porridge and couscous. It has enjoyed a period of renewed market interest in the west because it yields a gluten-free flour which may be used to produce gluten free pizza bases, breakfast cereals and Mexican style tortillas. It also caramelises very easily and is suitable as a coating for browning fried foods.

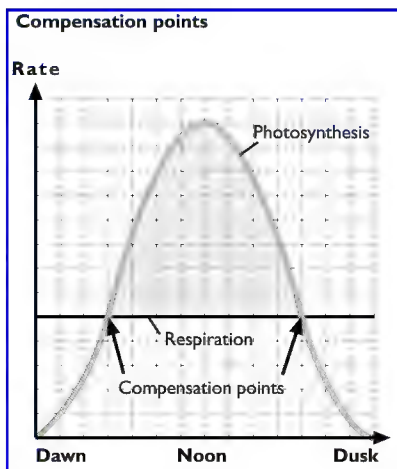


CONTROLLING THE ABIOTIC ENVIRONMENT

Human control of the abiotic environmental factors affecting productivity

A permanent increase in plant dry mass only occurs when the amount of carbohydrate produced by photosynthesis exceeds the amount oxidised in respiration and photorespiration. Remember that respiration is continuous over the whole 24 hours, whilst photosynthesis and photorespiration can only occur in the light, and it is the difference between these processes which determines plant productivity. The zero position at which the rate of photosynthesis and respiration are the same is referred to as the **compensation point**.

Productivity is measured as the gain in plant dry mass per square metre of land surface, but it is important to take into account the ratio between the leaf area of a crop and the land space it occupies. This ratio is called the **Leaf Area Index** or **LAI**, and it is calculated as the total leaf area per square metre of land. In the early stages of crop growth when new leaves are developing the LAI is small, but as more leaves are formed, it rapidly increases. Some leaves will shade each other, and in mature plants, older leaves die back as new ones are produced. For this reason it is rarely possible for a crop to use more than 95% of the available light.



Light intensity

The efficiency of light energy conversion into carbohydrate in crops depends not only on the amount of light received, but must also take into account losses through respiration and photorespiration (see above). The upper leaves of a plant tend to be illuminated at a level above the light saturation point for most plants. For a typical temperate crop, crop yields expressed as dry matter production relative to total incident light energy represent a less than 1% conversion of the solar energy input into chemical energy.

Productivity can be increased significantly by using crops bred to achieve high short term growth rates by maximising their percentage light utilisation. Crop research has also focussed on reducing losses by photorespiration, or increasing the availability of carbon dioxide which, under natural conditions, is normally the limiting factor.

Temperature

The ecological distribution of plants, their seasonal vegetative and reproductive cycles, and day to day carbohydrate assimilation, are all influenced, to a greater or lesser degree, by the ambient temperature. Temperature, like carbon dioxide and light, can be a limiting factor in photosynthesis, but it is more accurately regarded as an indirect influence, because above 15-20°C, it affects the rate of respiration more strongly than the rate of photosynthesis.

The most important factor affecting productivity is the average night temperature. 'Dark respiration' uses up twice as much assimilated carbohydrate at 25°C than at 15°C, for example, so cool nights may promote better growth.

Temperature has other indirect effects on the rate of photosynthesis, notably on stomatal opening but this is only in the extreme range. At temperatures above 30°C stomata generally close, probably as a result of higher carbon dioxide levels created by increased respiration.

Most temperate crops give their best production between 10 -15°C, but the optimum for other regions varies from 5 °C in some arctic species to 30°C for a tropical crop like maize.

Carbon dioxide

Carbon dioxide forms a very small proportion of the atmosphere, just 0.04% by volume, but it is estimated that crop plants fix an average $160\,000\text{ kg.km}^{-2}.\text{year}^{-1}$. Although the quantity of carbon dioxide in the atmosphere remains relatively constant, local concentrations around a crop can range from 0.015-0.1%. Photosynthesis depletes the available carbon dioxide, particularly in a closed environment like a greenhouse. The most important source for replenishment comes from the respiration of soil organisms carrying out decomposition of dead organic matter (soil respiration). In a fertile soil with adequate dead organic matter and soil organisms, the carbon dioxide produced by soil respiration and released to the air will exceed photosynthetic requirements.

The amount of carbon dioxide available to crops is limited by the frequency and degree of opening of its leaf stomata and also by the **microclimate** which surrounds the individual leaves. Around each leaf a **boundary layer** of still air tends to reduce the concentration gradient for diffusion. Air turbulence is important to replace the layer of air which surrounds the leaf canopy. Windy conditions help to overcome the problem of carbon dioxide depletion in a field of photosynthesising maize in high light intensity.

Glasshouse production and Hydroponic Systems

An understanding of the various environmental factors controlling plant growth has led to the development of soil-free or **hydroponic** plant culture systems. Hydroponic systems provide a 'state of the art' illustration of how a detailed knowledge of the effects of abiotic factors can be applied to plant production. By maintaining all the limiting environmental and nutritional factors at their optimum level in controlled glasshouses it is possible to achieve a high yield in a small space and a short time period.

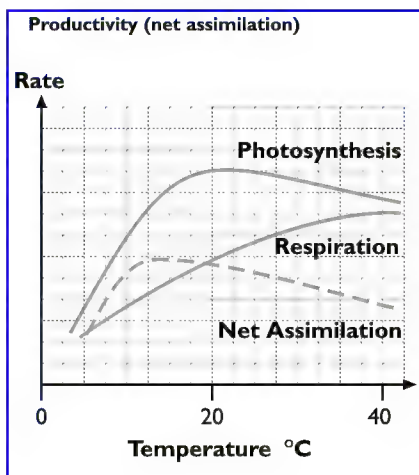
Hydroponic systems can be set up anywhere regardless of soil conditions and climate, even, as the Japanese have shown, within urban supermarkets to provide fresh clean vegetables with zero transport and minimum packaging costs. Pest and disease problems are minimal, and weeds do not have a chance to enter the system. With no large scale mechanical cultivation and no release of agrochemicals and fertilisers into the environment, hydroponic crops are environmentally friendly, particularly in systems where the water and nutrients are recycled.

Balancing these obvious benefits are the large set-up and skilled labour costs. It must also be considered that although disease is largely eliminated, if a crop does become infected, the process can be rapid and devastating.

The most common hydroponic system for growing tomatoes, cucumbers and lettuces is the **Nutrient Film Technique**, where plants are grown within gullies made from plastic film. Typically, the gully is constructed from a sheet of plastic, 65-80cm wide, coated black on the inside and white on the outside. It is stapled together between the plants and gently inclined so that the nutrient solution drains into catchment pipes which re-circulate it via a catchment tank and associated pumping system.

If plant roots require extra aeration, the nutrient solution may be supplied intermittently (in bursts).

Problems may arise when vigorous root growth prevents circulation of fluids.



◆ CHECKPOINT SUMMARY

- ◆ Productivity is measured as the gain in plant dry mass per square metre of land surface. This reflects the relative rates of photosynthesis and respiration
- ◆ The abiotic environment includes light intensity, temperature, and carbon dioxide concentration
- ◆ Increasing light intensity increases the rate of photosynthesis up to a certain point, the light saturation point, past which there are no further gains as the result of light stimulated photorespiration
- ◆ Temperature, like carbon dioxide and light, can be a limiting factor in photosynthesis, but it is more accurately regarded as an indirect influence, because above 15-20°C, it affects the rate of respiration more strongly than the rate of photosynthesis
- ◆ The most important factor affecting productivity is the average night temperature. 'Dark respiration' uses up twice as much assimilated carbohydrate at 25°C than at 15°C, for example, so cool nights may promote better growth
- ◆ Most temperate crops give their best production between 10 -15°C, but the optimum for other regions varies from 5 °C in some arctic species to 30°C for a tropical crop like maize.

Controlling the glasshouse environment

Seasonal and climatic changes in light intensity, wavelength and duration may be compensated for in a variety of ways. Where day length influences flowering, e.g. chrysanthemums, it is controlled by the use of artificial light and/or partial shading.

The optimum temperature for tomatoes and lettuces is 18-22°C. It is important to lower the night temperature by 6-12°C in order to minimise dark respiration. Control is achieved through thermostatic monitoring and the use of heaters, vents, shades and, where necessary, coolers. The temperature directly affects humidity so fine mist sprays are linked to the same control system.

Glasshouse plants, being confined to an enclosed space, quickly use up the available carbon dioxide and so it is very important to supply this by some external means. Where heating is the norm, a hydrocarbon burner may be used to supply carbon dioxide. Alternatively, dry ice or gas cylinders may be used.

Insect pests are potentially disastrous in the glasshouse environment. They are sometimes controlled by pesticides introduced via the fine mist spray system, although biological control agents are increasingly common. In the absence of chemical insecticides, bee hives may be kept in glass houses providing natural pollinators for fruits such as tomatoes, cucumbers, and strawberries. Otherwise pollination must be achieved manually.

FERTILISERS

Crop productivity is affected by the availability of inorganic nutrients in the soil. Plants take up inorganic elements from the soil solution in the form of anions (negatively charged ions) and cations (positively charged ions). Some elements, like nitrogen, phosphorus, and potassium are required in relatively large quantities (up to 150 kg per hectare). These are called **macronutrients**. Others such as zinc and copper are needed in trace amounts only and these are termed **micronutrients**. Some make up structural components of plant cells; nitrogen, for example, is essential for amino acid synthesis, phosphorus occurs in DNA and ATP, and magnesium forms part of the chlorophyll molecule. Other elements such as potassium are involved in enzyme systems. Iron is a component of the cytochromes which make up the respiratory electron transport chain; calcium controls membrane permeability, preventing leakage of other ions from plant cells.

The mineral nutrients most frequently applied to soils as fertiliser are nitrogen, phosphorus and potassium. They are often combined in what is known as an **NPK mixture**. The relative amounts of each component and the timing of the application can influence both the development and yield of a crop. In a maize crop, for example, nitrogen is important at the end of the growing season when the protein content is being laid down in the seed.

Nitrogen is the most important limiting nutrient because without it, plants cannot make proteins. Nitrogen exists in the soil as nitrate (NO_3^-), nitrite (NO_2^-) and ammonium (NH_4^+) ions. All of these can be directly utilised by plants but by far the most commonly available form is the nitrate ion (NO_3^-). Nitrate is released as a result of the activity of nitrifying bacteria such as *Nitrosomonas* and *Nitrobacter* which oxidise ammonium compounds utilising the energy derived from these reactions to manufacture organic food compounds.

◆ CHECKPOINT SUMMARY

- ◆ The quantity of carbon dioxide in the atmosphere remains relatively constant at 0.04%, but local concentrations around a crop can range from 0.015 - 0.1%, and under natural conditions in daylight carbon dioxide is the rate regulating (rate limiting) factor for photosynthesis
- ◆ By maintaining all the limiting environmental and nutritional factors at their optimum level in controlled glasshouses it is possible to achieve a high yield in a small space and a short time period
- ◆ An understanding of the various environmental factors controlling plant growth has led to the development of soil-free or hydroponic plant culture systems.
- ◆ Where day length influences flowering as in the case of chrysanthemums it is controlled by the use of artificial light and/or partial shading
- ◆ The optimum temperature for tomatoes and lettuces is 18-22°C. It is important to vary the day and night temperature by 6-12°C in order to minimise dark respiration. The temperature directly affects humidity so fine mist sprays are linked to the same control system
- ◆ Glasshouse plants, being confined to an enclosed space, quickly use up the available carbon dioxide. Where heating is the norm, a hydrocarbon burner may be used to supply carbon dioxide. Alternatively, dry ice or gas cylinders may be used.

Nitrate ions (being negatively charged) are not held by the negatively charged clay particles in the soil, and are very susceptible to being washed out of the soil (leaching). This causes variations in the soil concentration even on a daily basis. By contrast, ammonium ions (NH_4^+) are held by the negative charges on soil particles and are directly usable by plants.

Phosphorus is an essential component in three major biomolecules, ATP, nucleic acids and phospholipids. It is taken up by plants in the form of H_2PO_4^- or HPO_4^{2-} ions. These ions do not tend to move freely in the soil and become fixed in compounds not available to plants. Up to 80% of phosphate fertiliser, normally applied in the form of 'super phosphate' (P_2O_5), can be wasted in this way, so it is sometimes introduced with the seed as it is sown, a practice called **banding**, ensuring that the nutrient is available where the roots actually grow.

Potassium is absorbed in large quantities by plant roots, reaching concentration levels up to ten thousand times that of the soil solution in some *Brassica* (cabbage and rape) species. Potassium ions have a role as activators in enzyme systems, notably in protein synthesis, and they play a key role in the opening and closing mechanisms of stomata.

Organic and inorganic fertilisers

Organic fertilisers should be seen as complementary to, rather than an alternative to, the inorganic (chemical) fertilisers described above. The main source of organic nutrients is animal waste, either in solid form mixed with bedding materials (farmyard manure, FYM) or as liquid or semi-liquid (slurry). The quality of animal manure depends on the type of animal, what it is fed on, and how much bedding is used. It is ploughed into the soil, yielding a slow release of nutrients. More importantly, it improves soil structure by binding small particles together and retaining water.

Modern intensive animal farming methods tend to use less bedding, and mechanical devices shift the dung to storage tanks in liquid or semi-liquid form. Liquid manures are potentially very hazardous to the environment. They have a high biological oxygen demand (BOD) causing eutrophication, and far from improving soil structure, they tend to create anaerobic conditions by flooding the soil air spaces. For these reasons strict regulations apply to their use where flooding may occur near rivers.

Other sources of organic nutrients include sewage sludge, seaweed, waste material from the cotton and wool industries and dried blood and bone meal. Sewage sludge whilst cheap and easily available, may contain pathogens and heavy metals and is best avoided altogether. The use of other sources depends upon the relative local costs and benefits.

Green manure

Green manure is the term used to describe the practice of growing and ploughing into the soil a green (non-seed) crop such as white mustard or clover. Green crops may be used as 'cover crops' which cover the soil during the winter months, preventing the erosion and leaching of valuable inorganic nutrients.

◆ CHECKPOINT SUMMARY

- ◆ Harvesting removes nutrients from the soil by preventing the nutrients in the crop from returning to the soil as a result of decomposition
- ◆ Crop productivity is affected by the availability of inorganic nutrients in the soil, which are supplemented by the addition of fertilisers
- ◆ The mineral nutrients most frequently applied to soils as fertiliser are nitrogen, phosphorus and potassium. They are often combined in what is known as an NPK mixture
- ◆ Nitrogen is the most important limiting nutrient
- ◆ Up to 80% of phosphate fertiliser, normally applied in the form of 'super phosphate' (P_2O_5), can be wasted by becoming fixed in compounds not available to plants
- ◆ Organic fertilisers should be seen as complementary to, rather than an alternative to, the inorganic (chemical) fertilisers described above. The main source of organic nutrients is animal waste
- ◆ Organic fertilisers improve soil by binding small particles together and retaining water
- ◆ In modern intensive animal farming methods the dung is stored in tanks in liquid or semi-liquid form. Liquid manures are potentially very hazardous to the environment. They have a high biological oxygen demand (BOD) causing eutrophication and tend to create anaerobic conditions by flooding the soil air spaces
- ◆ Green manure is the term used to describe the practice of growing and ploughing into the soil a green (non-seed) crop such as white mustard
- ◆ The main nutrient which leaches out of the soil to pollute rivers and lakes is nitrate, which acts as a fertiliser in the aquatic ecosystem causing 'super feeding' or eutrophication
- ◆ Eutrophication causes unnaturally rapid growth of plants, occasionally forming algal blooms on the surface of the water
- ◆ The increase in plant material, over a period of time leads to increased amounts of dead organic matter which become food for aerobic bacteria. Bacteria have a high biological oxygen demand (BOD) and consequently starve the other oxygen uses of essential supplies. The highest oxygen users, notably fish and certain insect larvae suffer population decline and extinction
- ◆ In extreme cases conditions become anaerobic and the ecosystem collapses.

Leaching and Eutrophication

The main nutrient which leaches out of the soil in any quantity to pollute rivers and lakes is nitrate. The effect of nitrate in aquatic ecosystems is to fertilise the water causing 'super feeding' or **eutrophication**. This causes unnaturally rapid growth of plants, occasionally forming **algal blooms** on the surface of the water. The increase in plant material, over a period of time leads to increased amounts of dead organic matter which become food for aerobic bacteria. Bacteria have a high biological oxygen demand (BOD) and consequently starve the other oxygen users of essential supplies. The highest oxygen users, notably fish and certain insect larvae suffer population decline and extinction. In extreme cases, especially in lakes and ponds, conditions become anaerobic and the aquatic ecosystem collapses. In running water there is an 'oxygen sag' immediately downstream of the source of pollution, which slowly recovers further downstream. The quantity of nitrate applied as fertiliser to the soil should be calculated on the basis of **optimum use** by the crop and not simply on maximum crop yield.

The CONTROL of BIOTIC FACTORS (PESTS and COMPETITORS)

Weeds

Weeds compete aggressively with crops for water, light and mineral nutrients and generally show better tolerance to limited supplies. They are characterised by high rates of photosynthesis, and a rapid development of roots and leaves, so they gain access to their needs more quickly than their neighbours. Weeds tend to be the first colonisers of waste and disturbed ground, more resistant than other plants to environmental extremes, and more tolerant of changing conditions. Some, like couch grass, secrete toxic or growth retardant substances called **allelochemicals** to fight off the competition.

It is very difficult to isolate the specific effects of individual weeds in a crop because several weed species compete with the crop, and with each other simultaneously for light, water, and minerals, and the resources being competed for, are often in short supply (limiting), and interrelated. One principle, however, is universal. The closer the requirements of crop and weed, the more damaging the competition, and the more difficult it will prove to control the weed. This is most obvious when the weed and crop belong to the same plant family, for example, wild oat (*Arvena fatua*) in cereal crops, fat hen (*Chenopodium album*) in sugar beet and charlock (*Sinapsis arvensis*) in oilseed rape. If the relationship is very close there is a further danger of hybridisation between weed and crop to produce an even stronger competitor.

The degree of competition between weed and crop depends largely upon the relative timing of their vegetative and reproductive cycles. Harvest yields will be affected most seriously if the growth and reproductive stages of the weed coincide with those of the crop.

Insects

The success of insects as competitors for human food may be explained by two main biological features. One is the hard exoskeletal material, **chitin**, which can be fashioned into so many different feeding accessories that no plant part is immune from attack. Consider the mandibles and maxillae of the locust and aphid. In the locust, the mandibles form pincer like cutting tools whilst the maxillae are designed to guide a leaf blade into position for biting. In the aphids, both the mandibles and maxillae are sharpened and lengthened into a piercing hypodermic structure which penetrates the tissues of plants gaining direct access to the sugary sap in the phloem. The other biological feature of insects which accounts for their phenomenal success is their ability to survive and multiply rapidly in the harshest of conditions.

Insecticides - the Chemical Approach to Control

A general distinction can be made between chemicals which act by penetrating the outer cuticle of insects, termed **contact insecticides**, and those which are ingested, the so-called **stomach poisons**.

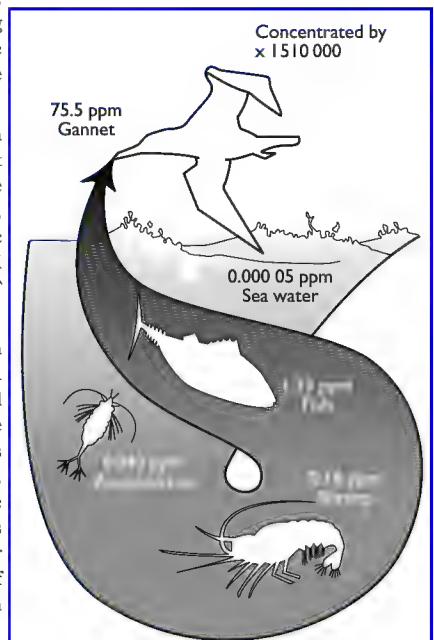
Of the older pesticides, Pyrethrum is a natural product extracted from several species of chrysanthemum (although synthetic versions have been developed) and is a contact insecticide which penetrates the cuticle of the insect as it rests against a sprayed surface.

Paris green (calcium acetoarsenite), was the first chemical insecticide used successfully on a large scale, is a stomach poison which is ingested as the insect feeds on sprayed vegetation.

Organochlorines were first developed as pesticides in the 1930s. They combine two important properties. They are **persistent**, remaining in a stable and active form for periods exceeding a year, and **residual**, accumulating in the fatty tissues without being excreted or broken down. Organochlorines destabilise the nerve membranes and tend to inhibit the respiratory enzyme cytochrome oxidase.

DDT was the first to be highlighted as having adverse effects on higher organisms quite unrelated to the target pest. The persistent and residual properties which made it so effective in eradicating the malarial mosquito and combating the insect pests of fruit trees, caused it to accumulate in natural food chains, particularly aquatic ones. Birds, such as pelicans and eagles, at the top of long food chains, diminished in number as the multiplied doses of DDT affected the shell forming mechanism in their reproductive tracts.

Organophosphates were first developed in the 1940s as poison gases for use in the Second World War. They include **parathion** and **malathion** which are still among the most widely used of all insecticides. They are easily formulated into sprays which coat the surface of leaves, and may penetrate into the epidermal tissues giving a **semi-systemic** action. When ingested by an insect pest, organophosphates act by combining with, and inactivating, the enzyme cholinesterase at nerve synapses. Cholinesterase is responsible for the removal from the synapse of the transmitter substance acetylcholine after each nerve impulse, so the result of organophosphate ingestion is the accumulation of acetylcholine in the synapses, leading to paralysis.



What are the alternatives? There are three other important approaches, namely biological control, cultural control, and the development of resistant crops, none of which alone forms a viable strategy for maintaining food levels. They should not be considered as alternatives to each other but as complementary strategies in a balanced and integrated approach to pest management.

Biological Control

Biological control involves the use of other organisms, usually predators or parasites, to reduce the numbers of a pest population. A good example is provided by the tiny parasitic wasp, *Encarsia formosa*, which has been used to control whitefly in commercial glasshouses since 1926.

Whitefly (*Trialeuroides vaporariorum*) is a sap sucking insect closely related to aphids, which owes its name to an opaque layer of wax coating its body and wings, giving it a white appearance. It is not a native British species, having been accidentally introduced from tropical America, but it thrives in glasshouses, and is very destructive of tomato, cucumber and chrysanthemum crops, not least because of its ability to transmit viral infections.

The tiny wasp, also tropical in origin, lays its eggs in young immature whiteflies referred to as 'scales' killing them before the young wasps eventually hatch out. The wasp was bred extensively for sale to glasshouse growers throughout the 30s and 40s but was made largely redundant by the advent of DDT. Biological control of whitefly using *Encarsia* enjoyed a revival in the 1970s as problems of pest resistance to chemical insecticides were recognised.

The development of a new biological control agent starts with a research programme to identify, collect and breed natural pest enemies in their natural habitat. Once sufficient numbers have been bred (and here it is important to get as much genetic variety as possible) the agent undergoes a quarantine phase in which it is assessed in laboratory conditions for its reproductive rate, climatic resistance, appetite for its pest prey, and potential threat to other organisms. After this it must undergo five years of field trials. The total cost of development is less expensive than the development of a new insecticide. Biological agents also have the advantage of reaching their target organisms in all conditions and in places inaccessible to sprays. They keep reproducing once established in the field, and there is little chance of the pest developing resistance.

However, there are a number of problems associated with the use of biological control methods. In order to maintain a healthy breeding population of *Encarsia* it is necessary to keep the temperature of the glasshouse within very strictly controlled limits. If it is too cool, then the wasps will not breed effectively. Conversely, in warm conditions the wasps become overactive and wipe out all the young whitefly, thereby denying the next generation of its sole food supply.

You can see from this example how biological control programmes depend on carefully managed population dynamics. Despite the checks referred to earlier, there are also environmental risks resulting from a possible switch by the predator or parasite to alternative and beneficial insect prey species. New diseases are constantly imported with non-native plant introductions, many of them notifiable under strict government regulations. The penalties for delay in treatment are high, so growers are encouraged to seek rapid chemical remedies.

◆ SUMMARY OF CHEMICAL CONTROL

The chemical control of insect pests poses four well publicised hazards:

- ▼ Insecticides may be directly poisonous (toxic) to man
- ▼ Insecticides may accumulate in food chains and have serious, even fatal, effects on top carnivores (including man)
- ▼ Insecticides may reduce populations of beneficial insect species.
- ▼ The use of insecticides encourages the increase of resistance in pest species.

Cultural Control

In uncultivated land, plants, insects, and fungi co-exist within a balanced ecological system. Population numbers fluctuate, but no individual organism acquires the status of a pest. It is important to recognise that pests are created by agriculture, and the problem becomes more acute the more intensive the methods employed.

The practice of growing crops in single stands or **monocultures** accelerates the growth of particular insect populations. In uncultivated land, insects are scattered as widely as their host plants. Their populations tend to reach equilibrium at much lower densities because they encounter a greater diversity of natural enemies, and compete with each other more acutely. Intensive farming both eliminates many natural enemies, and ensures a plentiful food supply. Under such conditions, the pest population soars rapidly, reaching artificially high densities.

Cultural control is the term used to describe the application of different techniques of cultivation with the aim of reducing yield loss through pests and competitors.

Where crops are widely spaced, it is advantageous both for weed control and for moisture retention to provide ground cover between the plants. In small banana plantations this is done by **mulching** which involves covering the exposed soil surface after tillage with rotting banana leaves. A more high-tech (and costly!) version of mulching can be seen in 'pick-your-own' strawberry farms, where plastic sheeting is used to cover the ground between the rows of strawberries. A better option altogether is to fill the spaces by planting a secondary crop, for example cowpeas in maize, or groundnuts in coffee. This practice, called **intercropping** is both economically and environmentally sound.

Crop rotation is an obvious method of cultural control of insect pests. It is calculated that the maize, wheat, red clover rotation harbours up to 50 insect pest species, but no more than 3 are common to all three crops. This may be combined with the planting of mini-hedgerows, a practice referred to as **strip farming**, providing wild vegetation which will encourage the growth of natural predator populations. An ingenious method of cultural control has been used in Canada to protect wheat crops against a stem-boring sawfly. A small strip of brome grass is planted around the crop and this attracts the egg-laying female flies to deposit their cargo before reaching the wheat. When the young larvae hatch out, they tend to eat each other and little damage results to the crop. The brome grass acts as a decoy or **trap crop**.

Hygiene

Regardless of cultural practices or chemical control methods, a high degree of preventative hygiene is essential in order to avoid contamination of a crop with weed seed or fungal spores brought in on machinery or irrigation water. This is particularly important in the battle against fungal diseases. Machinery and storage facilities are disinfected after each harvest, and crop debris should be cleared, either by ploughing in or, ideally, by burning.

Integrated Crop Management

Attitudes to pest control have changed considerably over the last thirty years, not simply because of a wider consciousness and concern for environmental protection. The main change is a philosophical one, focussed by the rather late realisation that it is not possible to eliminate pest species. Instead of searching for the ultimate weapon, therefore, a more rational approach would concentrate on ways of managing pest populations.

Pest management depends upon systematic and long term evaluation of the biology of weeds, insects, and fungi, and that of their natural enemies. Computerised predictive models of infestation and crop yield assessment may be devised, and linked to weather information giving farmers early warning of potential economic damage. In this way, all the methods outlined above may be brought into play in a more coordinated manner, ensuring that war is only waged in cases where the cost of control is less than the cost of the loss in yield.

However, a new age and an additional defensive strategy has opened up with the development of transgenic resistant plants. It is now increasingly possible to manipulate all aspects of the crop, including crop protection.

Integrated crop management embraces chemical, biological, and cultural control, as well as the manipulation of plant genetics. The environmental and public health impact of any particular strategy must be assessed, both in isolation and in combination with other factors. Education of food consumers is also very important. Informed customers no longer accept indiscriminate spraying of fruit and vegetables with chemical pesticides, and they already tend to reject products from countries where such practice is common, regardless of attractive qualities such as the colour, size, price, and shelf life of the product.

◆ CHECKPOINT SUMMARY

- ◆ Weeds compete with crops for water, light and mineral nutrients and show better tolerance to limited supplies. They have high rates of photosynthesis, and development, so they out compete crop plants
- ◆ Weeds tend to be the first colonisers of cultivated ground, more resistant than other plants to environmental extremes, and more tolerant of changing conditions
- ◆ The closer the requirements of crop and weed, the greater the competition, and the more difficult to control the weed
- ◆ If related there is a further danger of hybridisation between weed and crop to produce an even stronger competitor
- ◆ Harvest yields are affected most if the growth and reproductive stages of the weed coincide with those of the crop
- ◆ The success of insects as competitors for human food may be explained by the wide variety of feeding methods, and their ability to survive and multiply rapidly in the harshest of conditions
- ◆ Contact insecticides penetrate the exoskeleton of insects, and stomach poisons are swallowed by the insect
- ◆ Organochlorines are persistent, remaining in a stable and active form for periods exceeding a year; and residual, accumulating in the fatty tissues without being excreted or broken down
- ◆ Organochlorines destabilise the nerve membranes and tend to inhibit the respiratory enzyme cytochrome oxidase
- ◆ The chemical control of insect pests poses four well publicised hazards. Insecticides may be directly poisonous (toxic) to man. Insecticides may accumulate in food chains and have serious, even fatal, effects on top carnivores (including man). Insecticides may reduce populations of beneficial insect species. The use of insecticides encourages the increase of resistance in pest species
- ◆ Biological control involves the use of other organisms to attack the pest, cultural control involves cultivation techniques which reduce conditions favourable to the pest, and the development of resistant crops involves the breeding-in of resistance to the pest
- ◆ None of these alone forms a viable strategy for maintaining food levels
- ◆ They should not be considered as alternatives to each other but as complementary strategies in a balanced and integrated approach to pest management.

2.7

Biotechnology and the manipulation of reproduction in humans and domestic animals

Contents

Reproduction and its hormonal control

- ▼ The development of ovarian follicles and corpora lutea and changes in the uterine endometrium during the sexual cycle in a female mammal
- ▼ The hormonal control of the female sexual cycle in a mammal
- ▼ The roles of FSH, LH, oestrogen and progesterone
- ▼ The detection and significance of oestrus in a named farm animal

Manipulation and control of reproduction

- ▼ The use of extracted and synthetic hormones as contraceptives and in controlling human infertility
- ▼ In domestic animals, the role of hormones in:
 - producing large numbers of embryos for transplanting
 - synchronising breeding behaviour in sheep
 - increasing milk production
- ▼ The moral and ethical issues associated with using biotechnology to manipulate reproduction

REPRODUCTION and its HORMONAL CONTROL MANIPULATION and CONTROL of REPRODUCTION

Introduction

In both male and female mammals all aspects of sexual reproduction, including gamete production and mating behaviour, is under hormonal control. In both sexes interactions occur between reproductive hormones produced by the pituitary gland under the brain, and those produced within gonads (ovary and testes) which function to ensure optimum fertility. Understanding these interactions, particularly within the female, has provided many opportunities for human intervention in the process of reproduction.

Sexual cycles in the female mammal

In the sexually mature female mammal regular sexual cycles occur, during which one or more female gametes (ova, singular ovum) develop inside follicles and are released from the ovary, offering the chance of fertilisation if mating occurs. The general term for such cycles is the oestrus cycle. Oestrus cycles really consist of two closely linked cycles which are synchronised by hormonal interactions. These are:

- ▼ the **ovarian** or follicular cycle in the ovary which is concerned with the development and release of ova.
- ▼ the **uterine** cycle which is concerned with the development of the endometrium (uterus lining) to ensure the highest chance of implantation of a fertilised ovum.

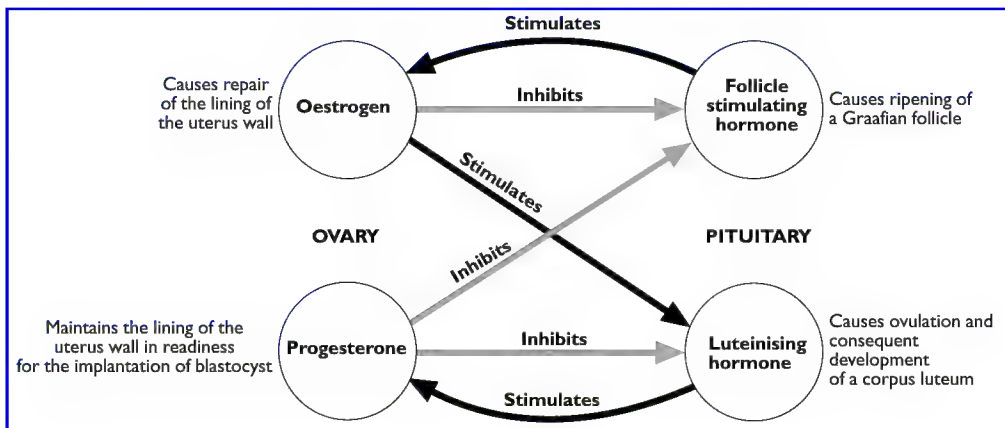
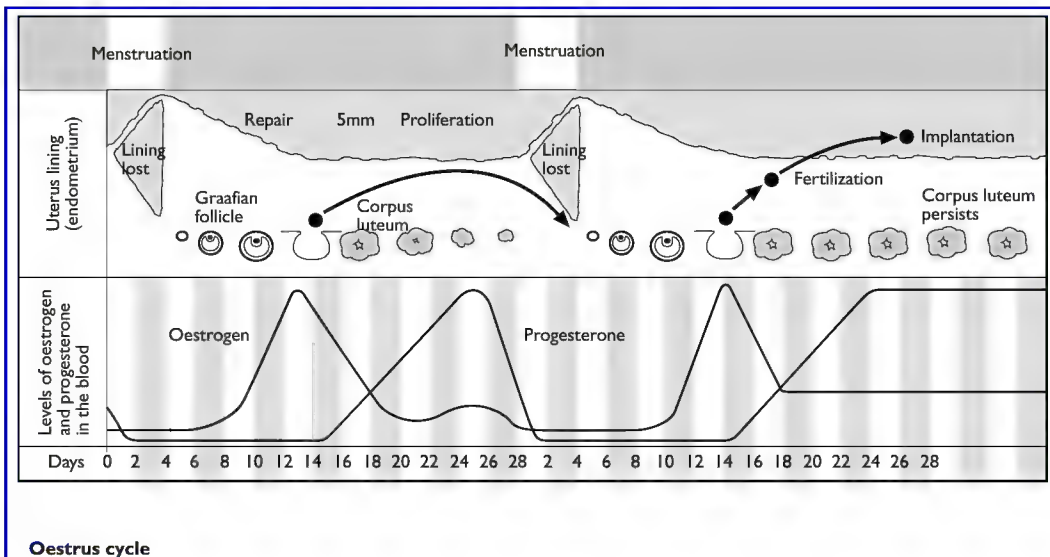
In the human female each cycle is approximately 28 days long. Ovulation which usually involves the release of a single ovum, occurs around the 14th day. The endometrium reaches a peak of thickness on day 21 and begins to break down on around day 28. This tissue, consisting of blood and cell debris is lost via the vagina during a menstrual period (menstruation). One cycle follows directly on from another throughout the year assuming that fertilisation does not take place.

Hormonal control of the oestrus cycle

The sexual cycle relies on interactions between four main hormones: the ovarian hormones **oestrogen** and **progesterone** and the anterior pituitary hormones **Follicle Stimulating Hormone** (FSH), and **Luteinising Hormone** (LH). Although timings of the cycle vary between species the same basic pattern holds true. This can be summarised as follows.

- a) FSH is released from the anterior pituitary and travels in the bloodstream to the ovaries where it stimulates the development of several follicles (primary oocytes). One or more of these will eventually mature into an ovarian (Graafian follicle) containing the ovum (secondary oocyte)
- b) FSH stimulates the production of oestrogen from the follicles within the ovaries. This oestrogen in turn stimulates the proliferation of the uterus lining (endometrium), replacing cells lost during the previous menstrual period in humans (or, in the case of animals, those cells which were broken down and absorbed). At this point oestrogen inhibits the secretion of FSH (negative feedback control).
- c) Oestrogen levels increase until the mid point of the cycle when they bring about a peak of LH production from the anterior pituitary causing ovulation (the release of the ovum (pleural ova) secondary oocytes from Graafian follicles). A small peak of FSH production also occurs at the time of ovulation.
- d) LH causes the empty Graafian follicle to develop into a corpus luteum which begins to secrete progesterone and oestrogen.
- e) Progesterone stimulates further development of the endometrium, in particular an increase in the density of blood vessels and the secretion of mucus and fluid from glands in the lining. This is sometimes known as the secretory phase and its function is to prepare the uterus in case the newly released ovum is fertilised.

- f) The other function of progesterone is to inhibit the production of both FSH and LH and hence the development of further follicles.
- g) If fertilisation does not occur the corpus luteum degenerates and secretion of progesterone and oestrogen falls. This fall causes the uterus lining and some of its blood vessels to break down. In humans this is lost during menstruation, whilst in other mammals it is reabsorbed. Decline in progesterone levels causes the inhibition of FSH and LH to be removed and a new cycle can begin.
- h) If fertilisation does occur then the corpus luteum does not degenerate but continues to secrete progesterone and oestrogen, maintaining the uterus lining and preventing further follicles from developing. After about 12 weeks of pregnancy in humans and corresponding times in other mammals the developing placenta takes over the secretion of progesterone and oestrogen.



Timing of sexual cycles

The length and details of the oestrus cycle varies amongst other mammals. Cycles may be as short as 4-5 days in the rat, extending to 21 days in the cow. In many wild animals, such as deer, only one cycle occurs per year which is associated with a particular breeding season probably triggered by environmental factors including changes in day length (photoperiod). Other animals with distinct breeding seasons such as the sheep may have several cycles within a given season whilst some such as cows and pigs resemble humans in having continuous cycles. This is known as being polyoestrus. Many large mammals (cow, horse) resemble humans in the release of a single ovum per cycle, whilst others such as the cat and rat may release as many as ten.

Oestrus and its significance

Oestrus is a change in the behaviour or appearance of a female mammal which occurs around the time of ovulation, indicating sexual receptiveness to males. It may involve the secretion of chemicals known as pheromones, swelling or change in colouration of the genital region or distinctive behavioural patterns, as discussed below. The human sexual cycle shows several unusual features which distinguishes it from the cycles of other mammals. Chief amongst these is the absence of oestrus, humans have the unusual feature of being sexually receptive at all times, regardless of the phase of the cycle.

In farm animals, such as cows, pigs and sheep, the presence of particular signs of oestrus which are detectable by humans, allows intervention in the process of reproduction. This is of great significance in modern farming where efficient breeding programmes are needed to allow the farm to exist as a profit-making business.

For a variety of reasons, including economic and safety reasons, many farms do not own male animals such as bulls, rams, stallions, or boars. Instead they may pay for the services of a male belonging to another individual, or, increasingly commonly, they may buy in semen with which to artificially inseminate the female animals. The use of semen (which is collected and stored in carefully controlled conditions to ensure high sperm quality and absence of sexually transmitted diseases) has many advantages over the use of natural breeding with a male animal. One of the most significant advantage is the ability to select and obtain semen from a male animal whose offspring are known to have specific desirable qualities such as high milk yield and quality, high meat quality, attractive appearance, and docility.

If artificial insemination (A.I.) or indeed mating, is to be used effectively then a farmer must be sure that the female animal is in oestrus before it is attempted. Insemination at other times will not result in pregnancy.

Signs characterising oestrus in the cow, pig and sheep are listed below. Careful observation by farmers, often several times per day, is necessary to spot these signs. Individual animals differ in the extent to which signs of oestrus are shown: some will show no signs, whilst others will show some or all of the signs.

The Cow

- ▼ Cows attempting to mount other females and then standing still and allowing themselves to be mounted by other females is the best sign of oestrus. On farms this may be detected in the following way: a type of paint is applied to the top part of the tail which will be rubbed off if the cow is mounted by another cow.
- ▼ Increased excitability. The cow shows a restless pattern of behaviour with increased time spent walking around and grooming other cows and decreased time resting.
- ▼ The cow may make a bellowing noise.
- ▼ A swollen vulva

NB. A bull may detect oestrus by sniffing the genital region of the cow (he detects chemical signals). In response to this he will show a lip curling response known as the 'flehmen response'.

The pig (sow)

- ▼ The most reliable sign of oestrus is the demonstration of the 'standing reflex' when pressure is applied to the lower back of the sow by the farmer. This is a rigid posture with the back legs extended that a female would adopt during mating. It occurs most frequently when pressure is applied to the back in the presence of a boar or when the female can detect the sound or scent of a boar. Synthetic 'boar odour' aerosols and tapes of boar sounds can be used instead of a real boar.
- ▼ A red swollen vulva and production of clear mucus from the vagina
- ▼ Sniffing of the genitals of other pigs and making a roaring or grunting noise may also occur.

The Sheep (Ewe)

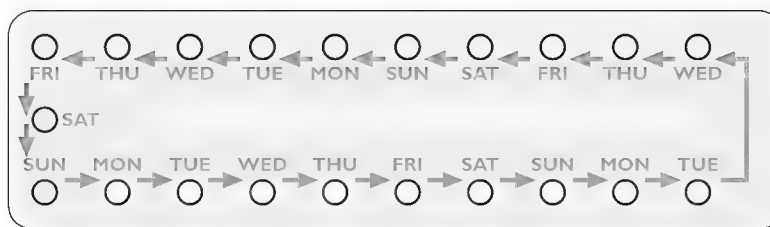
Detection of oestrus in the ewe is difficult since the sheep will only show signs if a ram is present. A farmer must therefore place a ram in with the sheep and watch the behaviour of both ram and ewes carefully. This ram may be a castrated animal and not therefore the male to be used for mating purposes. Under these circumstances oestrus is signalled by:

- ▼ Ewe standing firm and looking backwards, sometimes 'fanning' her tail (moving tail rapidly from side to side).
- ▼ Ram sniffing the genital region of the ewe, (suggesting the presence of chemical signals), curling his lip (flehmen response), pawing the ground and emitting a low pitched gurgling sound.

The control of reproduction: fertility and infertility.

The past century has seen a dramatic decline in the fertility of populations in most parts of the developed world and more recently within many developing countries. Fertility, in population terms, refers to the number of live births per 100 000 people and reflects the fertility of individual couples within that population. In Britain today each couple has, on average, 2 children compared to 6 or 7 a century ago. The main reasons for this is are an increase in the availability of contraception, associated with improvements in health (high chance of children surviving), education, and income.

Contraception can be defined as the prevention of conception (fusion of male and female gametes). Of the contraceptive techniques available to limit fertility, the most effective apart from abstinence or sterilisation, and most widely used, is the contraceptive pill (often called simply 'the pill'). First produced in 1951, and used but modified since that date, it works by interfering with the natural menstrual cycle of the human female and preventing ovulation. If used correctly it is over 99% effective in preventing pregnancy.



The most frequently used type of pill is known as the **combined contraceptive pill** which contains low doses of both of the steroid hormones oestrogen and progesterone in synthetic form. The effect of the pill is to prevent release of both LH and FSH via the negative feedback inhibition by oestrogen and progesterone. Without these hormones follicles do not develop in the ovary each month and are thus an ovum is not released. To achieve this effect the pill must be taken daily for 21 days in each 28 day cycle. In the 7 pill-free days which follow, the uterus lining breaks down in response to the withdrawal of progesterone, giving a type of light menstrual period. (In biological terms this is not strictly necessary but is built into the design of the contraceptive pill so that a semblance of normal cycles is achieved).

The contraceptive pill also exerts effects on other parts of the reproductive tract. Firstly it causes the mucus in the cervix region to thicken, making it more difficult for sperm to enter, and secondly it limits the thickness of the endometrium, making it more difficult for implantation of a fertilised ovum. If a progesterone only pill, the 'mini pill' is taken the effects are limited to these changes, inhibition of ovulation does not necessarily occur.

The development of other types of contraception based on hormones is continuing. One method which is already in use involves implanting tubes containing progesterone crystals under the skin which will release a constant low level of the hormone sufficient to reduce fertility. These may last for up to 5 years and have the advantage over the pill in that they do not need to be taken daily.

Treating infertility

Infertility, the inability to achieve pregnancy, easily or at all, is a growing problem in the developed world. Up to 1 in 8 couples in the UK may be affected. The causes are varied and can be associated with either the male partner, the female partner, or both. Causes include the long term consequences of some sexually transmitted diseases, genetic factors, physiological factors (including hormonal imbalance), psychological factors, environmental factors (exposure to some pollutants or to radiation), and increasing age of women trying to conceive. Whatever its cause infertility is a serious and distressing problem for many couples and it should be noted that whilst medical technology is improving the options for such couples, many of the treatments are lengthy, expensive and have low success rates. There are also a range of ethical and moral issues associated with some forms of infertility treatment which are discussed later.

In about 20 % of women who fail to conceive the underlying causes are hormonal. Hormonal imbalances can prevent the development of follicles or prevent the release of ova. In both cases treatment using synthetic or naturally occurring female hormones is highly successful in restoring fertility. A range of treatments is available most being based around stimulating the release of either FSH, LH or both. The first approach is to use a cheap synthetic anti-oestrogen drug called Clomiphene to stimulate the production of FSH and LH. If this is unsuccessful, FSH and LH themselves, may also be taken to stimulate the cycle at appropriate points. This treatment is more expensive since these hormones must be purified from the urine of post-menopausal women or produced by recombinant micro-organisms.

Where other infertility problems exist (including blocked oviducts in a woman and low sperm count of the male partner) treatment relies on various types of in vitro fertilisation (IVF). IVF involves the extracting of both ova and sperm from the body, encouraging fertilisation outside the body, and then transferring any resultant zygotes back into the uterus of a woman (who may or may not be the donor of the ova). In order to extract large numbers of ova to allow for several attempts at IVF, both FSH and LH are commonly used to encourage multiple ovulation.

A further use of female hormones is in hormone replacement therapy (HRT) used to treat some of the unpleasant symptoms of the menopause in older women. In such women, synthetic oestrogens are given to make up for the decline in natural production which occurs as the menstrual cycle stops. This has many benefits: it reduces some of the physiological and psychological changes that often occur at the menopause.

◆ CHECKPOINT SUMMARY

- ◆ Contraception can be defined as the prevention of conception (fusion of male and female gametes). The most widely used is the contraceptive pill
- ◆ The 'pill' works by interfering with the natural menstrual cycle of the human female and preventing ovulation
- ◆ The commonest used type of pill is the combined contraceptive pill which contains low doses of hormones oestrogen and progesterone in synthetic form
- ◆ The effect of the pill is to prevent release of both LH and FSH via the negative feedback inhibition by oestrogen and progesterone
- ◆ To achieve this effect the pill must be taken daily for 21 days in each 28 day cycle. In the 7 pill-free days which follow the uterus lining breaks down in response to the withdrawal of progesterone, giving a type of light menstrual period
- ◆ The contraceptive pill also exerts effects on other parts of the reproductive tract. Firstly it causes the mucus in the cervix region to thicken, making it more difficult for sperm to enter; and secondly it limits the thickness of the endometrium, making it more difficult for implantation of a fertilised ovum
- ◆ If a progesterone only pill, the 'mini pill' is taken the effects are limited to these changes: inhibition of ovulation does not necessarily occur
- ◆ The development of other types of contraception based on use of hormones are being investigated. One method which is already in use involves implanting tubes containing progesterone crystals under the skin which will release a constant low level of the hormone sufficient to reduce fertility
- ◆ In about 20 % of women who fail to conceive the underlying causes are hormonal. Treatment using synthetic or naturally occurring female hormones is highly successful in restoring fertility
- ◆ A range of treatments for infertility in the female are available based around stimulating the release of either FSH, LH or both
- ◆ Both FSH and LH are commonly used to encourage multiple ovulation for in vitro ('test tube') fertilisation.

HORMONES in DOMESTIC ANIMALS

Superovulation and embryo transfer

As discussed above, artificial insemination gives farmers the ability to select semen from male animals showing particular desirable characteristics, so improving the quality of his stock. Recently technology has become available to allow selection of breeding females in a similar, although slightly more limited way. This procedure involves manipulating the reproductive biology of females (usually cows) by superovulation and embryo transfer and involves the following stages:

- ▼ Cattle, or other farm animals are treated with hormone injections to stimulate the development and release of much larger numbers of ova than would naturally occur. As in humans, the main strategy involves using FSH and LH at appropriate times. Theoretically up to 50 ova may be released per cycle, but generally it is between 3 and 8.
- ▼ When ovulation occurs (detected by the signs of oestrus mentioned above, or by calculation of time since LH injection) the cow is artificially inseminated.
- ▼ The fertilised ova are allowed to develop for up to 7 days and are then washed out of the uterus before implantation occurs.
- ▼ The embryos are frozen and stored until required.
- ▼ Embryos are then introduced into the uterus of cows other than those from which they were obtained; they implant and develop until full term. This process is known as **embryo transfer**. It should be noted that whilst embryos may be implanted in females from the same farm they may also be sold for implantation in cows in different regions or countries.

By this process a single high quality cow may produce a very large number of offspring allowing the quality of a herd to improve very quickly. Estimates from the 1980s show that superovulation of a cow is possible every 2-3 months. Each time 6-7 normal embryos will result from artificial insemination, of which 3-4 will successfully develop into calves. This gives a total of 15-18 calves per donor female per year. As these calves grow up their qualities in terms of meat and milk production can be assessed allowing farmers to rapidly ascertain the best cattle to breed from.

There are variants on the type of embryo transfer described above and the reasons for performing it. One main aim is to use embryo transfer to establish twin pregnancies in cattle where twinning is uncommon. This can potentially double productivity. Twin pregnancies are most often obtained by transferring two embryos into a cow who has not been inseminated, although they may be added to an existing zygote in the uterus of a cow who has already been inseminated. In some cases the sex of embryos may be determined before transfer. This is valuable to farmers as it ensures that they can achieve desired ratios of male to female cattle (more males than females required for beef farming and vice versa for dairy). The embryos may also be split to produce clones (genetically identical individuals) before transfer.

Synchronisation of oestrus

The synchronisation of oestrus cycles in flocks of sheep (ewes) or herds of cows is a very desirable way of manipulating animal reproduction. The aim of this process is to ensure that all animals in the herd come into oestrus on approximately the same day and can be mated or artificially inseminated at the same time. This allows substantial savings for the farmer in terms of both time and money, as well as improving conception rates and ensuring that the entire flock will be producing offspring at the same time the following season. Synchronised oestrus is particularly desirable in ewes, as opposed to cows or sows for two reasons: firstly these animals have distinct breeding seasons and cannot become pregnant outside of this season and secondly, many flocks of sheep live in isolated hill regions. It is much more efficient to herd the sheep together to start treatment and then again for mating or A.I.

The process of synchronisation is based on the administration and then withdrawal of the female hormone progesterone. Progesterone inhibits FSH and LH secretion and hence prevents the development of follicles and the release of ova. The process is as follows:

- ▼ All ewes in a flock are treated with progesterone for 5-9 days. The hormone is administered in one of three ways: orally, via an implant under the skin, or via a sponge in the vagina.
- ▼ The progesterone is stopped in all ewes on the same day. This removes the inhibition of FSH and LH and allows follicles to start development in the ovaries of the ewes.
- ▼ Several days later the ewes come into oestrus.
- ▼ A ram is obtained and used to fertilise all the sheep at the same time. Alternatively A.I. may be used. This may ensure higher fertilisation rates, be quicker, prevent the spread of disease and allow semen to be chosen from a wide range of rams.
- ▼ All ewes who were fertilised produce lambs at approximately the same time.

In addition to its use in synchronising oestrus during the breeding season, the use of hormonal treatments has also allowed flocks of ewes to come into oestrus (synchronised) at other times of the year. In these cases progesterone is given as described above but an extra hormone, gonadotrophin, is required to stimulate FSH and LH secretion. Light stimulation is sometimes used as well since the breeding season is triggered by changes in light intensity. Such manipulation may be used to shift the timing of oestrus and hence of lambing to more suitable times of the year based on known or predicted environmental or economic conditions. In some cases, where environmental conditions permit, farmers may divide their flocks into two, having half mating in October and lambing in March and the rest mating in July and lambing in December.

Milk production in cows

Over the thousands of years of animal husbandry, cows have been selectively bred to give high milk yields. Traditional breeding techniques combined with the modern use of A.I. and embryo transfer and improved understanding of bovine (cow) nutrition and management has led to enormous increases in yield in domestic breeds compared to wild cattle. Even though a high yielding cow may produce over 40 litres of

milk per day (up to 12,000 litres/year) there is still an interest in further boosting production. One reason for this is that in many countries (including Britain), prices paid to farmers for milk are very low, forcing farmers to maximise productivity if they are to make a profit. One solution to this is to boost production using hormones.

The hormone BST, (bovine somatotrophin), is a pituitary growth hormone which occurs naturally in young cattle. Its function is to increase cell division, protein synthesis and growth. Trials with BST, which is now produced by genetically engineered bacteria, showed that injecting dairy cows with the hormone led to a 10-15% increase in milk yield, presumably through an increase in growth and activity of milk producing tissue in the udder. Some of the advantage was offset by the fact that cows injected with BST require more food, but a net profit still resulted. These early trials showed no apparent change in behaviour, health or reproductive capacity of the cows.

Although BST is approved for use in cows in the USA, the hormone is banned in the European Union. This decision followed public protest as well as further scientific investigations of cows treated with BST which did suggest some health problems, including reduced resistance to disease.

Moral and Ethical Issues associated with interventions in reproduction

Any intervention in the process of reproduction is likely to be considered unethical by some groups in society. One widely held view is that increased availability of contraceptives, and particularly of the contraceptive pill, encourages sexual promiscuity. In addition, some religions, including Roman Catholicism and Islam condemn the use of contraceptives of any kind, arguing that limiting procreation is against God's will. Such religions do indeed value large families, and in many parts of the world the large family does provide an effective support system for elderly people. In opposition, however, many other groups would point to the benefits to both individuals and societies that contraceptive technology has brought. In dramatically decreasing birth rates it has improved the physical and emotional well-being of women across the world. In limiting family size it has also helped to contribute towards improvements in child health and economic status in many regions.

Intervention in human reproduction to treat infertility (mainly that involving IVF) is also the subject of much debate. Although the number of people undergoing such treatments is very small compared to the numbers using contraception, the issues raised concern society as a whole. Increasingly issues concerning the rights of individuals to have children are becoming high profile legal cases, emphasising the fact that medical and scientific advances in this field must be matched by progress in the fields of ethics and law.

A major argument exists over the fate of spare embryos (or pre-embryos) that have been conceived by IVF. There is a case for using them in medical research; particularly research associated with improving understanding of infertility and the development of new techniques to treat it. Others, however, believe that embryos are new individuals and that this would be immoral or unethical. As with discussions surrounding abortion one of the issues here concerns the right to life and the ownership of the embryos.

One of the hazards of fertility treatment (including hormonal treatment and IVF) is the risk of multiple births (twins, triplets etc). Whilst in some cases this may be regarded as a bonus, such multiple births can place excessive strain on the parents, both financially and emotionally. In addition, with multiple pregnancies there is a much higher risk of the death of babies before and after birth. This may lead to a decision to have one or more fetuses aborted to ensure survival of at least one to full term. This, of course, raises its own ethical questions.

Genetic screening of embryos is commonly carried out before embryo transfer. With the potential of the human genome project to yield much more information about genes, both harmful and advantageous, there is a concern that screening may go beyond the bounds of detecting embryos with serious and life threatening diseases. It may, for example, lead to detection of milder disabilities, genetic predispositions to diseases, or even features of appearance. IVF has also raised fears of human cloning.

Concern has been expressed that embryos conceived via IVF may have an increased risk of some abnormalities. There is little evidence for this as yet, but it is possible that individuals who have reduced fertility through genetic causes but manage to conceive using IVF may pass on reproductive problems (and the distress associated with them) to their offspring.

If the discussion is extended to include forms of IVF where donors other than the parent are used then a range of additional issues arise. These are more specifically related to the rights, identity and psychological well-being of children produced in this way. Areas of concern include the question of whether and when to tell a child of his/her biological origins, whether the child is entitled to information about his/her biological parents, and whether such donor arrangements should be allowed in cases of single parents, older parents, lesbians or homosexual couples.

Another possible risk is that several children in an area may, unknown to them, have the same biological father (or more rarely mother) through use of gametes from anonymous donors. Recognition of the possible social and biological consequences of such half siblings meeting and entering a sexual relationship has led to the restriction on the number of sperm donations that can be made.

Moral and ethical concerns over the use of biotechnology in manipulating reproduction can extend to the use of such techniques in animals.

Using contraceptive measures in wild animal populations such as deer has received criticism. Whilst it may prevent culling of deer by shooting, the effects of suppressing fertility on the health of deer is unknown. In addition, contraceptives placed in foods may be eaten by other animals, perhaps reducing fertility in rarer species. In other circumstances the use of contraceptives may damage food chains.

The use of artificial insemination in combination with superovulation and embryo transfer raises further issues. This may lead to loss of genetic diversity as only certain individuals are effectively allowed to breed. The gene pool of domestic breeds would be reduced, perhaps with the complete loss of genes which might one day be desirable.



Student Text

Pathogens & Disease

FELTHAM PRESS

Pathogens and Disease

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Pathogens and Disease

3.1

Bacteria and Viruses as examples of pathogenic microorganisms

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Bacteria

- ▼ The sigmoid growth curve of a bacterial population. The effect of temperature and nutrient availability on the growth of bacterial populations.

Viruses

- ▼ The structure of the human immunodeficiency virus (HIV) and its replication

The association of microorganisms with disease

- ▼ Diseases as a result of pathogenic organisms penetrating any of the body's interfaces with the environment
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- ▼ Illustration of these principles by reference to:

Salmonella sp.

Mycobacterium tuberculosis

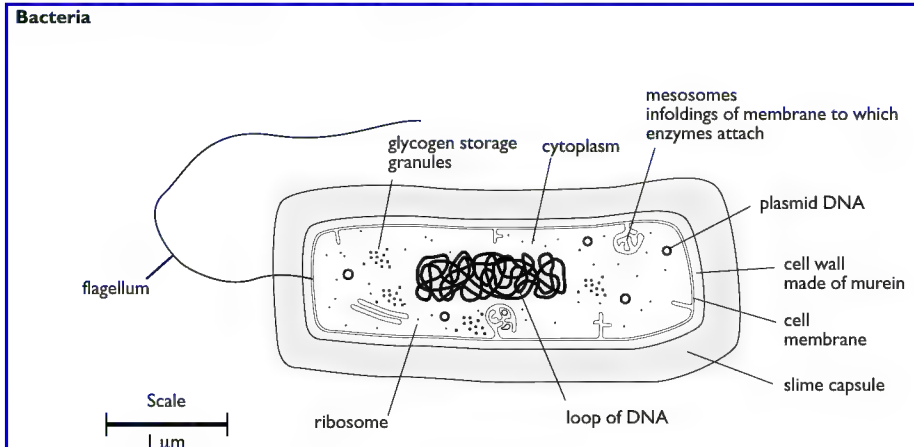
HIV

Introduction

Infectious or communicable diseases are caused by a range of organisms including viruses, bacteria, fungi, protoctists, arthropods, platyhelminthes and others which are collectively referred to as **pathogens**. The death toll from such pathogens is staggering: current estimates suggest that in 1999 diarrhoeal diseases caused by a range of bacteria killed almost 3 million people worldwide, HIV/AIDS (caused by a virus) another 2.6 million, tuberculosis (caused by a bacterium) over 1.5 million, and malaria (caused by a protoctist) a similar number. In many developing countries infectious diseases are still the most significant cause of death.

BACTERIA

Of the many thousands of species of bacteria only a relatively small proportion are human pathogens. Some common examples are *Salmonella enteritidis* a cause of food poisoning, *Mycobacterium tuberculosis*, the cause of tuberculosis (TB) and *Streptococcus sp.* a cause of throat infections. Other bacteria live freely in the soil, air or water, or mutualistically in or on animal hosts without causing them harm. Examples are forms of *Escherichia coli* (*E. coli*) which inhabit the large intestine of humans, and *Nitrobacter*, a soil dwelling bacterium which is involved in the nitrogen cycle.



Bacterial Growth

Bacteria reproduce asexually by binary fission (one cell dividing to form two identical daughter cells). The time taken for one cell to become two is highly variable, but it can be around 30 minutes. This doubling of the population every 30 minutes leads to rapid increases in numbers, and can explain why an individual may appear well one day and be severely ill or dead from a bacterial infection the next. The increase in numbers of a bacterial population is referred to as bacterial growth.

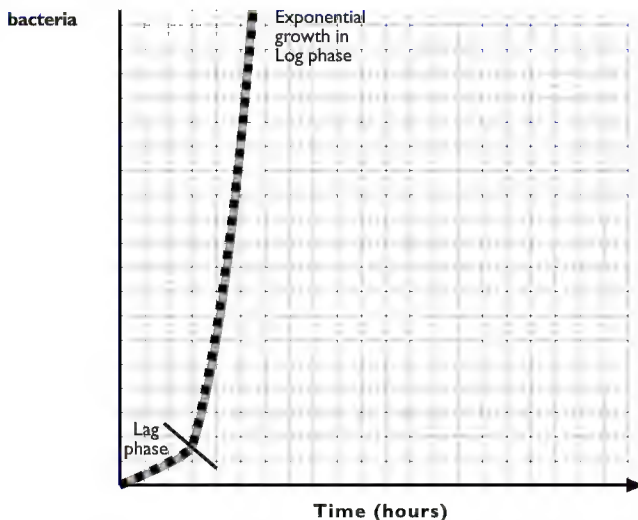
Colonies grown in a closed system, e.g. a petri dish, where there are no additions or removals of materials once growth has started, show a characteristic **sigmoid growth curve** when numbers of bacteria are plotted against time (sigmoid means curved like a capital S). Such curves can be derived from laboratory analysis of bacteria growing in a nutrient medium (a **bacterial culture**) which would require sampling the culture and counting the number of bacteria in a sample of equal size at regular time intervals. The curve has four main phases:

- ▼ **Lag phase.** In this initial phase the bacterial culture is getting started, but division is not occurring at a maximum rate. The bacteria are adapting to their new surroundings and possibly synthesising new enzymes to digest the nutrient medium.
- ▼ **Log phase.** This is the period of maximum growth. The population increases by doubling at intervals (e.g. 2->4->8->16->32->64 etc.). If the actual numbers of bacteria are plotted on a graph this phase would be represented by an increasingly steep curve. Usually, however the numbers are converted to logarithms to the base 2. In other words the power to which two must be raised to give that number, e.g. $4 = 2 \times 2 = 2^2$ and the log is 2; $8 = 2 \times 2 \times 2 = 2^3$ and the log is 3, etc. In mathematical terms, 3 is the log of 8 to the base 2. When the logs are plotted they produce a straight line. Thus instead of 2->4->8->16->32->64 etc. the figures would be 1->2->3->4->5->6 etc.
- ▼ **Stationary phase.** After a brief period of growth at a reduced rate, bacterial growth ceases and the numbers stay constant. Some bacteria are still dividing at this point, but an increased death rate prevents any overall (net) increase in numbers. Reasons for this stationary period include depletion of nutrients, depletion of oxygen, and accumulation of waste products.
- ▼ **Decline phase.** In this final part of the curve bacteria stop dividing and the death rate increases. Numbers may eventually fall to zero.

◆ CHECKPOINT SUMMARY

- ◆ Bacteria reproduce asexually by binary fission (one cell dividing by mitosis to form two identical daughter cells). The increase in numbers of a bacterial population is referred to as bacterial growth.
- ◆ The nature of this growth process is such that a characteristic sigmoid growth curve can be plotted with numbers of bacteria against time.
- ◆ The curve has four main phases:
- ◆ Lag phase in which the bacterial culture is getting started, but replication is not occurring at a maximum rate.
- ◆ Log phase which is the period of maximum growth. The population increases by doubling at intervals (e.g. 2->4->8->16->32->64 etc.).
- ◆ Stationary phase. After a brief period of growth at a reduced rate, bacterial growth ceases and the numbers stay constant.
- ◆ Decline phase. In this final part of the curve bacteria stop dividing and the death rate increases. Numbers may eventually fall to zero.
- ◆ In natural habitats such curves are unlikely to be found as ideal conditions for exponential growth and a habitat free of competitors or predators is rarely found.

Bacterial Growth Curve



This curve is produced in laboratory investigations of bacteria. In natural habitats such curves are unlikely to be found, as ideal conditions for exponential growth, and a habitat free of competitors or predators is rarely found. When microorganism are grown commercially, for example during the production of insulin in genetically engineered yeast or bacterial cells, then conditions are carefully monitored to ensure optimum growth.

Bacteria culture at 30°C

Time/h	Number of cells in millions	
	Living	Living & Dead
0	9	10
1	10	11
2	11	12
5	18	20
10	400	450
12	550	620
15	550	700
20	550	850
30	550	950
35	225	950
45	30	950

VIRUSES

Viruses are structures from 20-300 nm in size, which contain genetic material in the form of DNA **or** RNA (never both) and infect cells of other organisms (prokaryotic and eukaryotic). There is much debate as to whether they should be defined as living organisms since although they possess genetic material, they lack many of the basic features shown by all other living organisms, including the presence of a cellular structure. Viruses are incapable of reproducing themselves outside the environment of a host cell.

Whilst details vary, all viruses have similar structural features which include a central core of genetic material (DNA or RNA never both) and a capsid or protective protein coat around the core formed of units called capsomeres.

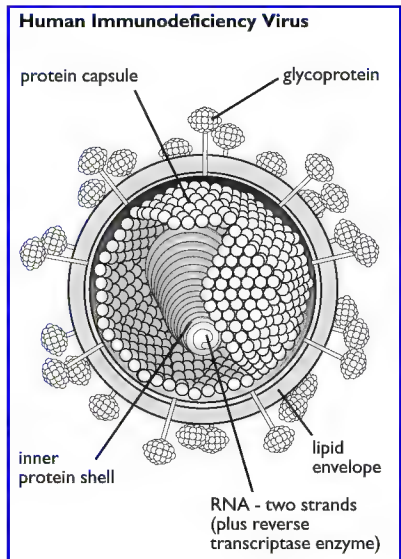
As a group, viruses cause an enormous amount of damage and death amongst organisms of all five kingdoms (bacteria, fungi, protoctista, plants and animals) yet each virus is very specific to one particular type of cell in one host species. In humans, viral diseases include chicken pox, measles, influenza, and HIV/AIDS.

Human Immunodeficiency Virus (HIV)

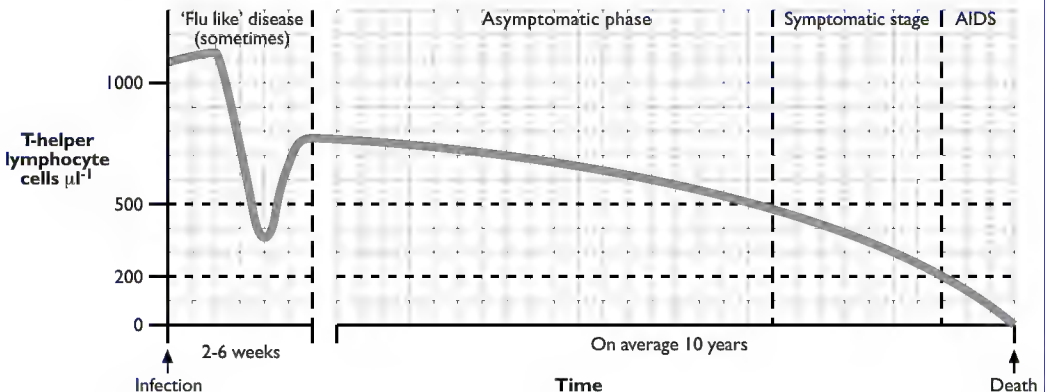
HIV is a virus which causes the disease known as AIDS (Acquired Immune Deficiency Syndrome). This disease is one of the fastest growing causes of death in the world, and is currently responsible for about 3 million deaths per year. HIV infects a type of white cell known as the T-helper lymphocyte or CD4 cell. In doing so it severely damages the immune system of infected people, making them highly susceptible to infection and death from a range of infectious diseases and cancers.

Structure of HIV

HIV belongs to a group of viruses known as **retroviruses**. The main feature of such viruses is the presence of RNA rather than DNA as the genetic material in the core of the virus. The HIV virus has two identical RNA strands. Also present is the enzyme reverse transcriptase, which allows single stranded RNA to be converted to double stranded DNA. The RNA is encapsulated in layers of proteins, glycoproteins and lipids.



Decline of T-helper lymphocytes in the course of an HIV infection



Replication of HIV

Like all viruses, HIV is incapable of independent replication. It relies on inserting its genetic material into the DNA of human T-helper cells and allowing these cells to manufacture new copies of the viral RNA and proteins necessary for assembling new viruses. There are several stages in this process:

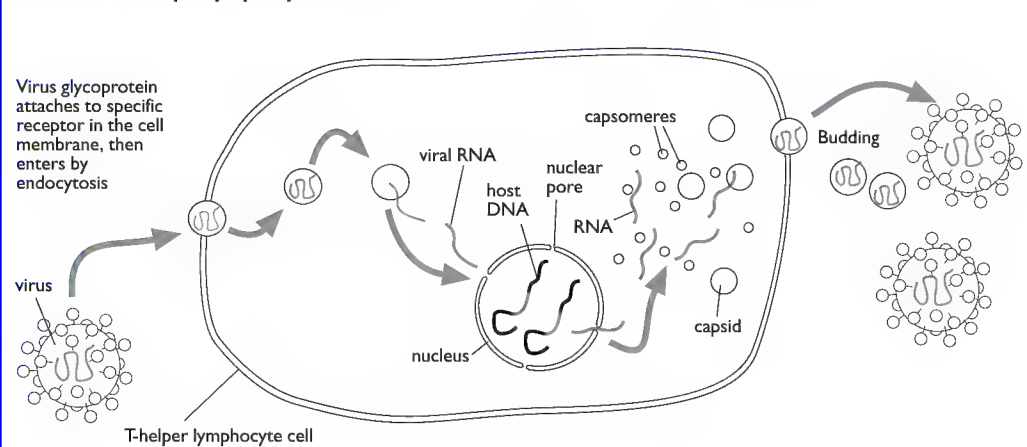
- ▼ HIV enters the body from an infected (HIV positive) person via body fluids such as blood or semen.
- ▼ It attaches to specific receptors on the cell membranes of T-helper lymphocytes.
- ▼ HIV enters the cell by endocytosis releasing its RNA and reverse transcriptase enzymes into the cytoplasm.
- ▼ The RNA strand is copied to form a double stranded DNA molecule using reverse transcriptase.
- ▼ The DNA copy inserts itself into the human DNA in the nucleus of the T-helper lymphocyte.
- ▼ After a variable period of time (latency period) the DNA is copied (transcribed) to make new viral RNA. At the same time, the viral proteins necessary for the capsid and envelope proteins are synthesised by the cell.
- ▼ New viruses are assembled from the RNA and proteins and leave the cell by exocytosis. During this process the viral envelope is constructed from the cell membrane of the host cell.

As with any virus, large numbers of new HIV viruses are made by a single cell, all of which may, in turn, infect new cells. Since HIV eventually kills the T-helper lymphocytes it has infected, one of the signs that an HIV infection has become active is a rapid decrease in the numbers of these cells. T-helper lymphocyte counts are used in the monitoring of HIV infection. Once the levels drop below 200 cells per μl of blood AIDS is diagnosed.

◆ CHECKPOINT SUMMARY

- ◆ Viruses are structures from 20-300nm in size, which contain genetic material in the form of DNA or RNA (never both) and infect cells of other organisms (prokaryotic and eukaryotic).
- ◆ Viruses are incapable of reproducing themselves outside the environment of a host cell.
- ◆ All viruses have similar structural features which include a central core of genetic material (DNA or RNA) and a capsid or protective protein coat around the core formed of units called capsomeres.
- ◆ HIV is a virus which causes the disease known as AIDS (Acquired Immune Deficiency Syndrome).
- ◆ HIV infects a type of white blood cell known as the T-helper lymphocyte or CD4 cell. In doing so it severely damages the immune system of infected people, making them highly susceptible to infection and death from a range of infectious diseases and cancers.
- ◆ HIV belongs to a group of viruses known as retroviruses. The main feature of such viruses is the presence of RNA rather than DNA as the genetic material in the core of the virus. The RNA is encapsulated in layers of proteins, glycoproteins and lipids.
- ◆ Like all viruses, HIV is incapable of independent replication. It relies on inserting its genetic material into the DNA of human T-helper cells and allowing these cells to manufacture new copies of the viral RNA and proteins necessary for assembling new viruses.

Infection of a T-helper lymphocyte with HIV



The ASSOCIATION of MICROORGANISM with DISEASE

Koch's postulates

Robert Koch, a nineteenth century bacteriologist was one of the first people to make a definite link between bacteria and specific infectious diseases. He is best known for his work in identifying the bacterium responsible for tuberculosis (*Mycobacterium tuberculosis*). Before Koch, microscopes were not powerful enough to allow bacteria to be seen, and thus many diseases which we now know to be caused by microorganisms were then said to be due to climatic, dietary or other causes.

Koch established three postulates (criteria) which must be fulfilled if an infective cause for a disease (such as bacteria or virus) is to be proven.

- ▼ The organism must be sufficiently abundant in every case to account for the disease
- ▼ The organism associated with the disease can be cultured (grown in the laboratory) artificially in pure culture
- ▼ The cultivated organism produces the disease upon inoculation into another member of the same species.

These are still as relevant today as in Koch's era although a few points should be noted. Firstly, some organisms are sparse and difficult to find in the body even though they are the definite cause of infection. Secondly, some organisms are difficult to culture in the laboratory, even though they have been proven to cause the disease. And thirdly it is unethical to deliberately infect humans with microorganisms so experimental animals must be used instead.

When considering Koch's postulates it is worth remembering that new diseases are still appearing whose cause needs to be discovered. HIV/AIDS, which first appeared in a significant number of cases in San Francisco in the early 1980s, is a good example of a disease whose cause was unknown for some time.

Entry of microorganism into the body

Disease causing microorganisms (pathogens) from the environment can enter the body in several different ways. Some pathogens have found complex modes of entry although most are opportunistic and enter when the body's basic defences against pathogens are breached.

The main points of entry are as follows:

- ▼ Through the skin when it is cut or damaged and thus ceases to act as a barrier to infection. In this case microorganism may enter the blood at the site of the wound. For example tetanus occurs when the bacterium *Clostridium tetani* enters a wound. HIV is most commonly transmitted during sexual intercourse when infected semen from one partner enters the blood of another via a cut or tear in the epithelium of the vagina or rectum.
- ▼ Through the mucous membranes of the respiratory tract when air containing droplets of infectious material are breathed in. This is the way in which *Mycobacterium tuberculosis* the bacterium which causes TB is transmitted. Cold and influenza viruses are also transmitted in this way.

▼ Through the digestive tract when food or water containing microorganisms is taken in. The bacterium responsible for cholera (*Vibrio cholerae*) enters via drinking water infected with faeces whilst *Salmonella enteritidis*, responsible for food poisoning enters when undercooked contaminated food, e.g. chicken meat or eggs are eaten. Microorganisms which cause infection via this route must be resistant to the acidic conditions of the stomach which offer protection against other microorganisms.

Other more complex means of entry of microorganisms into the body include transmission by insect vectors (such as malaria where the *Plasmodium* parasite is injected into the blood when the mosquito vector takes a blood meal) and direct entry through the intact skin (as in Schistosomiasis where the larval stage schistosome burrows in though the skin of the feet.) Both of these organisms are discussed in section 12.2.

How microorganisms cause disease

Once inside the body, microorganisms cause disease in a variety of different ways. In some cases the microorganisms actually damage and destroy the host cells. In other cases the microorganisms themselves do not directly cause harm, but produce toxins which are harmful. In still others it is the body's own immune response to the presence of the microorganism which produces the symptoms. These categories are not, however, mutually exclusive: some microorganisms will cause illness in all three ways.

Microorganism also vary considerably in the numbers of organisms required to cause illness: thus whilst very large numbers of *Salmonella enteritidis* bacteria are required to produce the symptoms of common 'food poisoning', only very small numbers of *Salmonella typhi* are required to cause the more serious symptoms of typhoid.

HIV infection is an example of a pathogen causing direct damage to human cells which in turn affect critical functions within the body. As discussed above, HIV only infects one type of cell: the T-helper lymphocyte, cells which play a vital role in the human immune system. Damage to these cells occurs during viral replication. As the infection progresses, more and more T-helper lymphocytes are infected and destroyed resulting in the condition of **Acquired Immune Deficiency Syndrome** (AIDS). Here the patient is unable to mount immune responses against common pathogens since the T-helper lymphocytes 'help' B and T lymphocytes to work.

Salmonella bacteria also cause direct damage to cells. When these bacteria arrive in the intestine they are taken up by epithelial cells. This process destroys the brush border of the microvilli, creating a characteristic 'ruffled' cell surface. These invaded cells then detach from the intestine wall, creating inflamed lesions. The effect of this is to cause the secretion of large amounts of watery fluid into the lumen of the gut, resulting in the characteristic diarrhoea experienced during an infection with *Salmonella*. It is interesting to note that species of *Salmonella* which do not cause illness are incapable of invading the intestinal lining.

Whilst mucosal damage may explain some of the features of *Salmonella* infections, some scientists believe that toxins produced by the bacteria are significant in causing disease. Many *Salmonella* species have been shown to produce toxins in vitro (in the laboratory)

but their role in the disease is not yet clear. The fact that such bacteria can cause illness within 6 hours of infection suggests to some that a preformed toxin (already present inside bacterial cells when they infect a person) is involved.

Clearer examples of the role of toxins in causing disease are illustrated by the cholera causing bacterium *Vibrio cholerae*, and members of the *E. coli* group of bacteria. *V. cholerae* bacteria themselves are relatively harmless but produce two harmful 'exotoxins' (a toxin released from the cell). One of these toxins causes a loss of chloride and hydrogencarbonate ions from the intestinal cells followed by an osmotic loss of up to 10 litres of water per day. Absorption of water and salts from the gut is also impaired. Together these facts explain the severe watery diarrhoea and death from dehydration which is characteristic of the disease.

Mycobacterium tuberculosis falls into the third basic category described above: that is the effect of the bacterium on the lungs (and indeed other parts of the body) is a result of an immune response by the body to the bacterium. In this case the body tries to destroy the invading bacteria, by walling them off in 'tubercles'. In doing so, however, inflammation and damage to the surrounding cells occurs. These damaged regions (lesions) may become hard or spongy, or liquefy leaving 'holes' in the lungs, sometimes damaging blood vessels. If many such lesions occur lung function is drastically reduced.

◆ CHECKPOINT SUMMARY

- ◆ Koch established three postulates (criteria) which must be fulfilled if an infective cause for a disease (such as a bacteria or virus) is to be proven.
- ◆ The organism must be sufficiently abundant in every case to account for the disease
- ◆ The organism associated with the disease can be cultured (grown in the laboratory) artificially in pure culture
- ◆ The cultivated organism produces the disease upon inoculation into another member of the same species.
- ◆ Disease causing microorganisms (pathogens) can enter the body through the skin when it is cut or damaged. HIV is most commonly transmitted during sexual intercourse when infected semen from one partner enters the blood of another via a cut or tear in the epithelium of the vagina or rectum.
- ◆ Through the mucous membranes of the respiratory tract when air containing droplets of infectious material are breathed in. This is the way in which *Mycobacterium tuberculosis* the bacterium which causes TB, and cold and influenza viruses, are transmitted.
- ◆ Through the digestive tract when food or water containing microorganisms is taken in. *Salmonella enteritidis*, responsible for food poisoning enters when undercooked contaminated food is eaten.
- ◆ Microorganisms cause disease in a variety of different ways. In some cases the microorganisms actually damage and destroy the host cells. In other cases the microorganisms themselves do not directly cause harm, but produce toxins which are harmful. In still others it is the body's own immune response to the presence of the micro-organism which produces the symptoms.
- ◆ HIV infection is an example of a pathogen causing direct damage to human cells which in turn affect critical functions within the body. As discussed above, HIV only infects one type of cell: the T-helper lymphocyte, cells which play a vital role in the human immune system.
- ◆ As the infection progresses more and more T-helper lymphocytes are infected and destroyed resulting in the condition of Acquired Immune Deficiency Syndrome (AIDS). Here the patient is unable to mount immune responses against common pathogens since the T-helper lymphocytes 'help' B and T lymphocytes to work.
- ◆ *Salmonella* bacteria also cause direct damage to cells. When these bacteria arrive in the intestine they are taken up by epithelial cells. These invaded cells then detach from the intestine wall, creating inflamed lesions. The effect of this is to cause the secretion of large amounts of watery fluid into the lumen of the gut, resulting in diarrhoea
- ◆ Toxins produced by bacteria may be significant in causing disease e.g. cholera
- ◆ *Mycobacterium tuberculosis* falls into the third basic category described above: that is the effect of the bacterium on the lungs (and indeed other parts of the body) is a result of an immune response by the body to the bacterium.

3.2.

Parasites and parasitism

Contents

- ▼ The principal adaptations of parasites to their way of life as illustrated by *Plasmodium* and *Schistosoma*
- ▼ These organisms studied in sufficient detail to illustrate:
 - their ability to survive in the hostile environment within the host
 - reduction of locomotory and other structures
 - modification of reproduction and the life cycle associated with infecting a new host

Introduction

A parasite is an organism which lives on or in a host, causing harm to the host whilst gaining benefits from it. These benefits include an unlimited supply of readily available nutrients and water, and constant optimum temperature. There are many different types of parasites of humans, which include bacteria, protoctists, viruses, fungi, arthropods, and platyhelminthes. Parasites are responsible for a large proportion of all human deaths in many developing countries, as well as having a very significant impact on domestic animals and plant crops.

Parasitic organisms often show a range of unique structural and functional adaptations to their lifestyle which distinguish them from non-parasitic relatives. Some key features include:

- ▼ Specialised reproductive strategies aimed at overcoming the unpredictability of finding new hosts, including producing vast quantities of offspring during phases of asexual reproduction.
- ▼ Modification of mouthparts, digestive systems and enzymes to allow attachment to the host and utilisation of host's food supply, blood or tissues.
- ▼ Features to resist attack by host enzymes or immune system. This may include having a tough protective cuticle to resist attack by host enzymes as well as strategies to evade the immune system.
- ▼ Reduction of unnecessary sensory organs and locomotory organs in the adult stages (especially in larger parasites) as they live in protected, optimum conditions surrounded by readily available nutrients and water.

The malarial parasite, *Plasmodium*.

Plasmodium is a single celled intracellular parasite of the Kingdom Protocista (Class Protozoa) which causes the disease malaria in humans. It infects approximately one third of the world's population and is responsible for over 1 million deaths per year. It has a complex life cycle which involves two hosts: the human and the female mosquito (*Anopheles sp.*), both of which are essential for its development. Whilst the human host is often severely affected by the parasite, the female mosquito host seems unaffected. Inside the human *Plasmodium* feeds on haemoglobin inside the red blood cells.

The life cycle of *Plasmodium* includes an asexual reproductive phase in the human (in liver cells and then red blood cells) and a sexual reproductive phase in the female mosquito. In spite of the complexity of the cycle and the relatively long time needed for its completion (2-3 weeks) the parasite is very successful in being transferred from one host to another, mainly as a result of the asexual phase producing enormous quantities of parasites (merozoite stage), and the sexual phase generating the genetic diversity which is essential for evading the host's immune system.

The parasite is transferred from human to female mosquito and female mosquito to human via the 'bite' of the female mosquito. When the female mosquito pierces the skin to take blood from a human, it secretes anti-coagulant saliva to prevent clotting of the blood. If the mosquito is infected with the malarial parasite, malarial parasites in the **sporozoite** form are injected into the human.

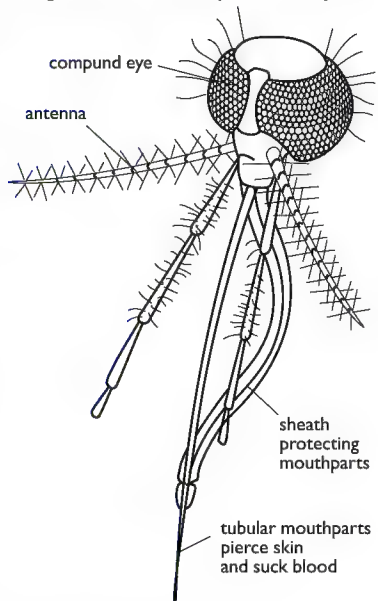
If the human is infected with the malarial parasite, the female mosquito takes up *Plasmodium* gametes in the blood on which the mosquito feeds.

Inside the human body *Plasmodium* is able to evade the immune system. Initially it lives inside the liver cells and subsequently inside the red blood cells, and cannot be targeted by the hosts immune system.

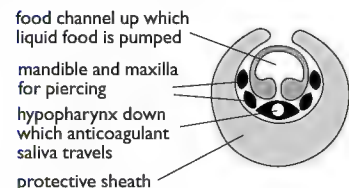
Life cycle

A malaria infection of a human starts when a female mosquito injects anticoagulant saliva containing young malarial parasites known as sporozoites into the blood of a human on which it is feeding. These parasites migrate to the liver cells, undergo asexual reproduction, develop into **merozoites** and then infect red blood cells. Here they also reproduce asexually by multiple fission producing huge numbers of additional merozoites which burst out of the red blood cells and cause the characteristic fever and other disease symptoms. While most merozoites enter new red blood cells for further rounds of asexual reproduction, some develop into **gametocytes** which can be taken up when another female mosquito feeds on the infected person's blood. Once inside the body of the female mosquito the gametes produced by these gametocytes fuse, and the resulting zygotes develop into sporozoites which move into the salivary glands of the female mosquito where they can be injected into another person. The cycle thus begins again.

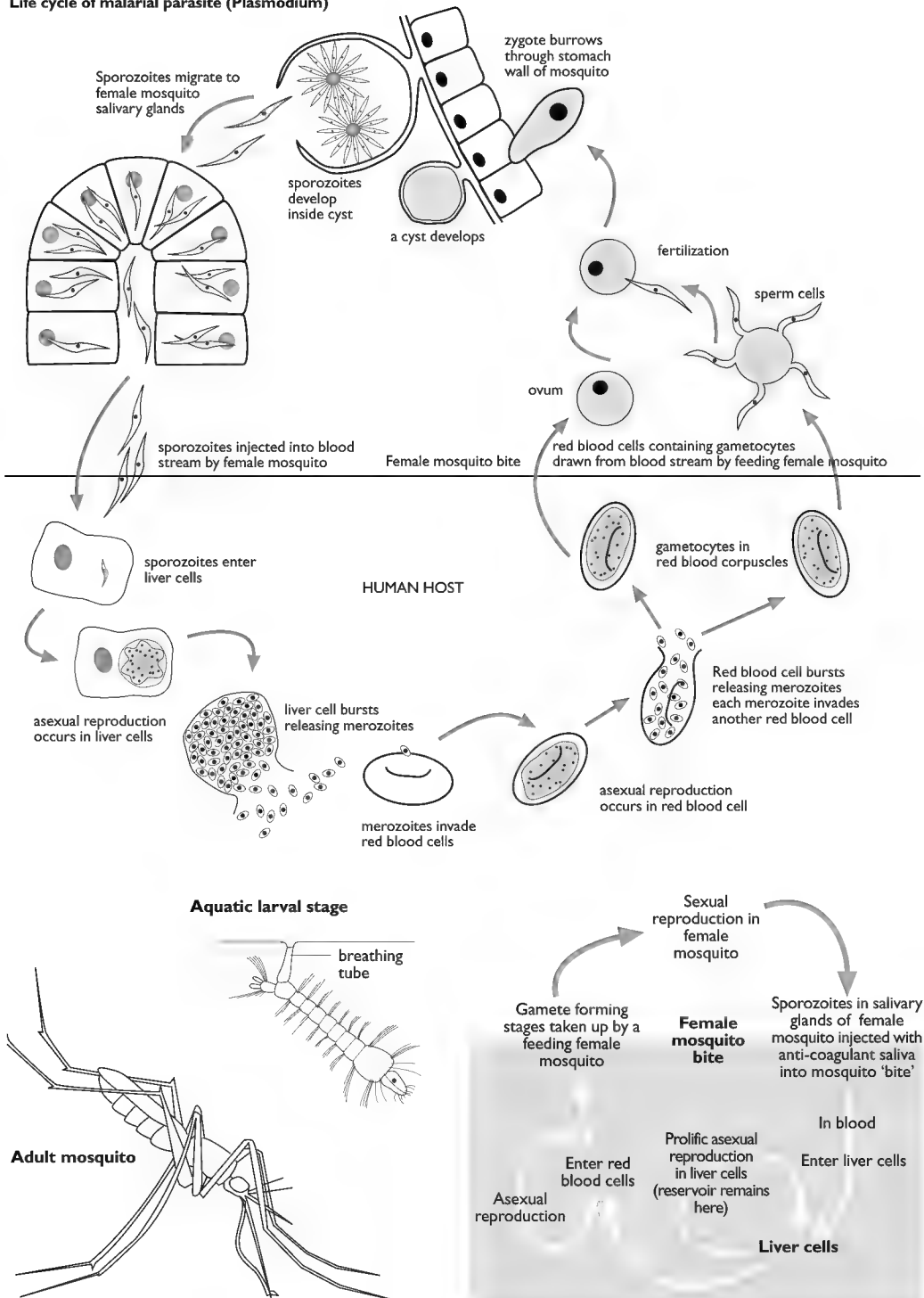
Diagram to show mosquito mouthparts



Section through mouthparts



Life cycle of malarial parasite (Plasmodium)



Schistosoma

Schistosoma is the name of a genus of parasitic flatworms (Phylum Platyhelminthes: Class Trematoda) which cause the disease schistosomiasis (formerly known as Bilharzia) in humans. This disease is thought to affect over 200 million people across the tropics. *Schistosoma* is an endoparasite, inhabiting the blood vessels of the bladder region (*Schistosoma haematobium*) or of the intestine (*S. mansoni* and *S. japonicum*). It attaches to the vessels via suckers and feeds directly on blood which it digests using a protease enzyme in its gut.

Like *Plasmodium*, *Schistosoma* has a complex life cycle involving two hosts. In this case certain fresh water snails are the non-human hosts. The complexity of the life cycle and relatively small chance of finding a new host is overcome by the enormous numbers of larvae produced during asexual reproduction.

Life cycle

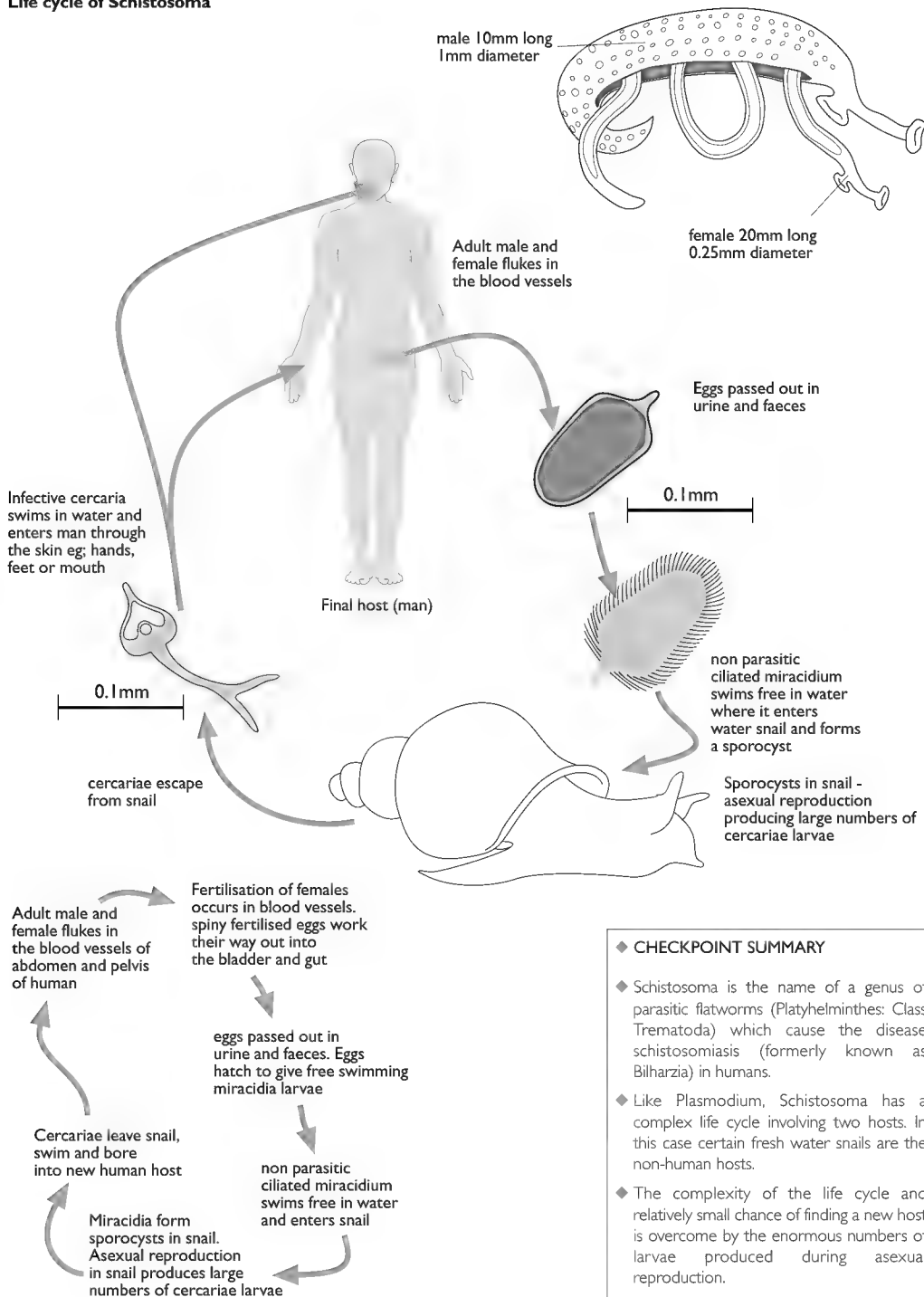
The life cycle of *Schistosoma* relies on water, and on the contamination of water by human urine and faeces. During the life cycle eggs pass out of an infected human via urine or faeces. They hatch into minute ciliated larvae (**miracidia**) which are capable of swimming until they find and burrow into the flesh of a water snail. Asexual reproduction occurs here producing free swimming larvae (**cercariae**) which burrow (aided by the secretion of digestive enzymes) into the human via the skin of the feet. The larvae then migrate either to the bladder or gut region where they may exist for many years. The importance of this asexual stage in generating vast numbers of parasites cannot be overemphasised: a single miracidium may give rise to 200,000 cercariae over a period of a few months.

Adult schistosomes exist as separate males and females which, are usually found attached to one another, to ensure mating and sexual reproduction. (A single human may only possess a few adult forms and if they did not attach to one another they might never meet to mate). The adult worms lack any means of locomotion and have no sensory organs as these are unnecessary in their habitat inside the host's body, where they are surrounded by nutrients in constant optimum conditions.

◆ CHECKPOINT SUMMARY

- ◆ A parasite is an organism which lives on or in a host, causing harm to the host whilst gaining nutritional and other benefits from it.
- ◆ Parasitic organisms often show a range of unique structural and functional adaptations to their lifestyle which distinguish them from non-parasitic relatives. Some key features include:
 - ◆ Specialised reproductive strategies aimed at overcoming the unpredictability of finding new hosts including producing vast quantities of offspring during phases of asexual reproduction.
 - ◆ Modification of mouthparts, digestive systems and enzymes to allow attachment to the host and utilisation of host's food supply, blood or tissues.
 - ◆ Features to resist attack by host enzymes or immune system. This may include having a tough protective cuticle to resist attack by host enzymes as well as strategies to evade the immune system.
 - ◆ Reduction of unnecessary sensory organs and locomotory organs in the adult stages (especially in larger parasites).
 - ◆ *Plasmodium* is a single celled intracellular parasite of the Protocist kingdom (Class Protozoa) which causes the disease malaria in humans.
- ◆ It has a complex life cycle which involves two hosts: the human and the female mosquito (*Anopheles* sp.), both of which are essential for its development. Inside the human *Plasmodium* feeds on haemoglobin inside the red blood cells.
- ◆ The life cycle of *Plasmodium* includes an asexual reproductive phase in the human (in liver cells and then red blood cells) and a sexual reproductive phase in the female mosquito.
- ◆ The asexual phase produces enormous quantities of parasites (merozoite stage), and the sexual phase generates the genetic diversity which is essential for evading the hosts immune system.
- ◆ When the female mosquito takes a blood meal from a human, malarial parasites are injected into the wound with the anti-coagulant saliva from the salivary glands, whilst *Plasmodium* gametes are taken up in the blood on which the mosquito feeds.

Life cycle of Schistosoma



3.3

Defensive functions of mammalian blood

Content

General mechanism of defence against disease

- ▣ Phagocytosis and the subsequent destruction of ingested pathogens
- ▣ The roles of thromboplastin, prothrombin, plasma enzymes, calcium ions and fibrinogen in blood clotting

Principles of immunology

- ▣ Definitions of antigen and antibody
- ▣ The essential difference between humoral and cellular responses as shown by B-lymphocytes and T-lymphocytes. The role of plasma cells and memory cells in producing primary and secondary response

Passive and active immunity

- ▣ Passive immunity
- ▣ Antibodies acquired through the placenta and via lactation and those acquired artificially
- ▼ Vaccination and immunisation
- ▼ Attenuated and dead microorganisms, and genetic engineering as the basis for vaccines

GENERAL MECHANISM of DEFENCE AGAINST DISEASE

In order to remain healthy and able to function properly, the human body must protect itself from disease-causing organisms, known as pathogens. Firstly it must aim to prevent pathogens from entering the body in the first place, and secondly it must be able to defend itself against them if they do enter. The first lines of defence are therefore the barriers to infection, which include the following.

- ▼ The impermeable skin, the surface layers of which are made up of many layers of dead, tough, keratin impregnated cells.
- ▼ The blood clotting process, which is rapidly activated to plug any wound and prevent pathogen entry.
- ▼ The mucous membranes of nose and respiratory tract which trap pathogens in sticky mucus, which is moved away from the delicate lining of the alveoli by cilia.
- ▼ Anti-bacterial enzymes in saliva, sweat and tears.
- ▼ Stomach acid (pH 2) which kills most pathogens entering with the food.

If pathogens do manage to pass through these barriers and enter the body tissues or blood, an immune system is necessary to attack and destroy these pathogens and so prevent serious disease or death. There are two branches of the immune system in operation within the body which co-operate with each other to some extent. These are:

- ▼ **Non-specific immunity** which is not targeted at specific types of pathogen but at foreign material in general. This involves phagocytosis and inflammation and is immediate.
- ▼ **Specific immunity** which uses B and T lymphocytes to target specific pathogens. Such responses are more complex and are often delayed.

Phagocytosis

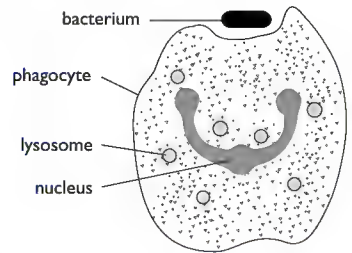
Phagocytosis is the process by which certain white cells (phagocytes) engulf and digest pathogens, other foreign material and dead or infected cells. These phagocytes are non-specific in their actions so will deal with any foreign material which they meet. One type of phagocyte (neutrophil) primarily engulfs bacteria, whilst another (macrophage), being larger, is capable of engulfing larger particles and cells including old or infected red blood cells.

The phagocytes are found in blood, lymph and the tissues, as they are capable of squeezing through the tiny gaps between cells in the walls of capillaries and entering the tissue fluid. This is important as it allows them to move to any body tissue which becomes infected with pathogens.

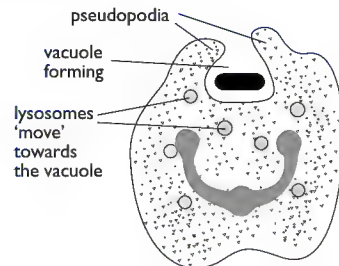
The process of phagocytosis in both neutrophils and macrophages involves several stages.

- ▼ The phagocyte comes into direct contact with the foreign particle or microorganism. In some cases phagocytes are attracted by chemical signals from damaged cells or invading bacteria and move towards them (chemotaxis). Some bacteria may become coated with opsonins (a type of antibody) which allows phagocytes to bind to them.

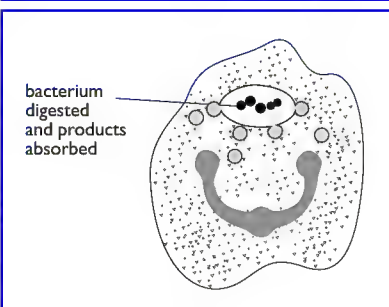
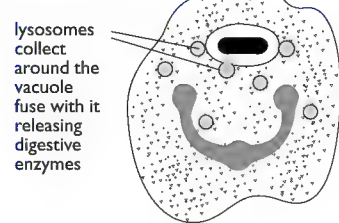
Phagocyte 'moves' towards bacterium



Vacuole forming



Vacuole formed



-
- ▼ The phagocyte begins the ingestion process. The cell membrane and cytoplasm move out forming 'pseudopodia' around the particle or microorganism.
 - ▼ The particle or microorganism is enclosed in a vacuole formed from the cell membrane of the phagocyte.
 - ▼ Lysosomes fuse with the vacuole and their digestive enzymes and other chemicals digest the particle or microorganism, and the products of digestion are absorbed by the phagocyte or released from the cell.

Blood clotting

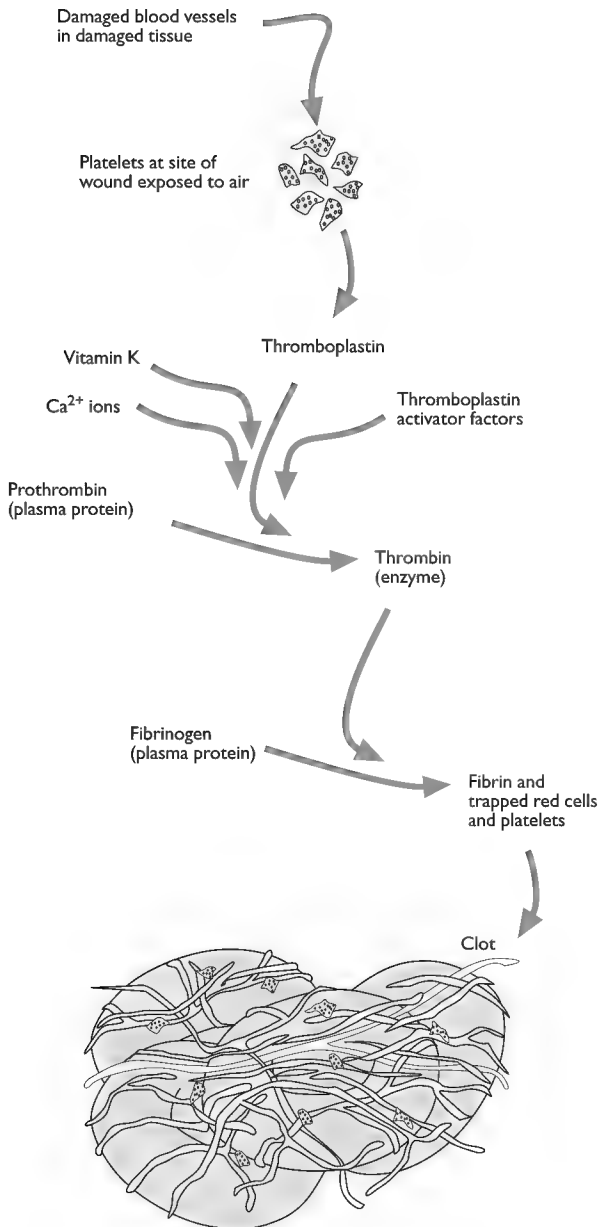
When intact the skin provides an excellent barrier against the entry of microorganisms that cause disease (pathogens). It is waterproof and impermeable to pathogens, whilst sweat and sebum contain anti-bacterial agents to further reduce the risk of infection. If, however, the skin is cut or damaged pathogens may easily gain entry to the tissues and blood and spread through the whole body. At the same time blood may escape from the wound, causing illness or even death. To limit the extent of both blood loss and infection mammals have evolved a blood clotting mechanism which allows the rapid formation of a hard clot or scab at the site of a wound.

The clotting process, which starts within seconds of skin damage, involves a series of stages, (a cascade) catalysed by chemicals known as 'clotting factors'. Many of these chemicals are enzymes and occur in the blood plasma in inactive forms until the clotting process starts: this is important as it prevents blood clotting inside blood vessels under normal conditions. The clotting process can be summarised as follows:

- ▼ Blood escapes from skin wound and comes into contact with air.
- ▼ Platelets and damaged tissues at the wound site release a number of substances including thromboplastin, several clotting factors (including factor VIII) and calcium ions
- ▼ Thromboplastin and other substances catalyse the conversion of inactive prothrombin to thrombin, an active protease enzyme. Calcium ions are necessary for this stage.
- ▼ Thrombin converts the soluble protein fibrinogen in the plasma to its insoluble form fibrin.
- ▼ The insoluble fibrin forms a dense mesh of cross-linked fibres which traps platelets and red blood cells around the wound. Platelets are activated and become sticky, attaching to the fibrin fibres forming a dense plug and also to the walls of any damaged vessels.
- ▼ Gradually the fibrin fibres pull the clot in tighter so that it completely plugs the wound.

Mutations in the genes coding for the plasma clotting factors result in a reduction in the efficiency of the blood clotting process. Collectively these conditions, which are inherited in a sex-linked manner, are known as haemophilia. In one of the most common types a gene responsible for the production of clotting factor VIII is faulty leading to the absence of this factor. In the past such individuals would often die from small wounds or internal bruises where the blood failed to clot. Now genetically engineered factor VIII produced in sheep is available for treatment of haemophiliacs.

Blood clotting



◆ CHECKPOINT SUMMARY

- ◆ If the skin is broken the blood clotting process is rapidly activated to plug the wound and prevent pathogen entry.
- ◆ The mucous membranes of nose and respiratory tract trap pathogens in the sticky mucus, which is moved away from the delicate lining of the alveoli by cilia.
- ◆ Anti-bacterial enzymes are found in saliva, sweat and tears
- ◆ Stomach acid (pH 2) kills pathogens in the food.
- ◆ Non-specific immunity is targeted at foreign material in general. Involves phagocytosis and inflammation and is immediate.
- ◆ Phagocytosis is the process by which pathogens, other foreign material and dead or infected cells are engulfed and digested by phagocytes. These phagocytes are non-specific in their actions.
- ◆ Specific immunity uses B and T lymphocytes to target specific pathogens. Such responses are more complex and are often delayed.
- ◆ Blood clotting involves a series of stages, (a cascade) catalysed by chemicals known as 'clotting factors'.
- ◆ Platelets and damaged tissues at the wound site release a number of substances including thromboplastin, several clotting factors (such as factor VII and factor X) and calcium ions
- ◆ Thromboplastin and other substances catalyse the conversion of prothrombin (inactive) to thrombin, an active protease enzyme. Calcium ions are necessary for this stage.
- ◆ Thrombin converts the soluble protein fibrinogen in the plasma to its insoluble form fibrin by hydrolysis.
- ◆ The insoluble fibrin forms a dense mesh of cross-linked fibres which traps platelets and red blood cells around the wound. Platelets are activated and become sticky, attaching to the fibrin fibres forming a dense plug and also to the walls of any damaged vessels.
- ◆ Gradually the fibrin fibres pull the clot in tighter so that it completely plugs the wound.

Principles of immunology.

Immunology is the study of the mechanisms by which foreign material entering the body is recognised and destroyed thus protecting the individual from harm. Such foreign material may be in the form of pathogens (disease-causing bacteria, fungi, viruses and protoctists), in the form of human cells from other individuals (as in blood transfusions or organ transplants), or, in the case of allergic reactions, in the form of molecules on common products like pollen and peanuts. In all cases, foreign or 'non-self' material is distinguished from 'self' material by the presence of antigens, and responses are specific to the type of antigen encountered.

Antigens

Antigens are defined as molecules, which, when foreign to an individual, stimulate an immune response by lymphocytes in the body, in particular the production of antibodies.

Antigens are often protein or glycoprotein molecules found on the cell surface membrane, but a range of other molecules, including inorganic molecules can also act as antigens. Antigens on the cell surface are important in cell recognition. Under normal circumstances the immune system of an individual will recognise all of his/her own cells as being 'self' owing to the familiar types of antigens present. Any cells (or other materials) with different (foreign) antigens are rapidly detected by white blood cells (leucocytes) known as **B-lymphocytes** and **T-lymphocytes** and targeted for destruction.

Types of Immune Response

An immune response, involves the recognition of foreign cells or particles and the initiation of a series of mechanisms to destroy them. There are two main types of immune response:

humoral response which is associated with B-lymphocytes and involves the production of antibodies;

cellular or cell-mediated response which is associated with the T-lymphocytes.

B-lymphocytes: The Humoral Response

On the cell surface membrane of B-lymphocytes are a number of specific antigen receptors or membrane bound antibodies. These are sites to which antigens on the surface of pathogens or other foreign material may become attached, leading to a sequence of events resulting in free antibodies being produced to destroy the foreign material.

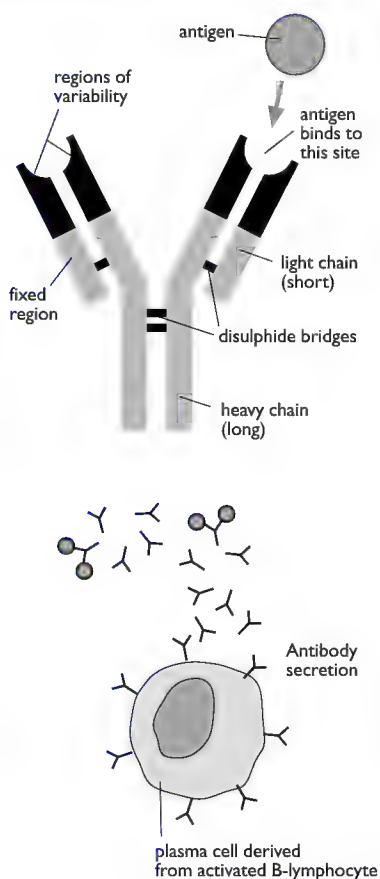
There are many thousands of specific types of B-lymphocyte and each is capable of recognising only one specific antigen. This allows highly specific antibodies to be produced in response to a vast range of antigens. For example, there is one type of B-lymphocyte which will attach to the bacterium causing TB and release antibodies against TB, and another type which attach to *Vibrio cholerae*, the bacterium responsible for cholera, and produce antibodies against it.

Antibodies are protein molecules known as immunoglobulins which are secreted by activated B-lymphocytes when a specific antigen is encountered. They are shaped like a 'Y' with the straight tail of the Y being known as the constant region since it is the same in every type of antibody. The forked part of the Y is known as the variable region and contains antigen binding sites which vary slightly in shape between each type of antibody. Antibodies do not directly destroy foreign material but target it for destruction by other branches of the immune system, including phagocytes.

Sequence of events in the humoral response against pathogens

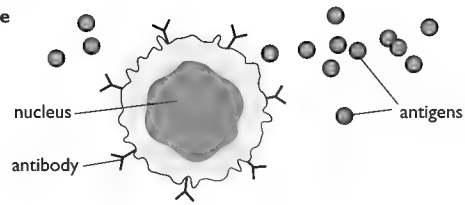
- ▼ Pathogen enters body.
- ▼ Antigens on surface of pathogen come into contact with their specific antigen receptor on a B-lymphocyte.
- ▼ Binding of antigen to antigen receptor activates the B-lymphocyte and causes it to divide by mitosis producing a clone of identical B-lymphocytes.
- ▼ Most of the B-lymphocytes turn into **plasma cells** and the rest turn into **memory cells**.
- ▼ The plasma cells begin to secrete specific antibodies against the pathogen concerned. Antibody molecules are secreted at a very high rate: up to 30 000 molecules per second.
- ▼ The antibodies attach to the antigens on the foreign material and lead to the destruction of it in one of three ways.
- ▼ By coating pathogens and causing them to stick together in such a way that they can then be engulfed by phagocytes (another type of white cell).
- ▼ By binding to the pathogens at sites which interfere with their activities. For example, by covering the protein coat of a virus and preventing it from attaching to host cells.
- ▼ By stimulating the release of other blood components called '**complement**' to destroy the pathogen.
- ▼ Once the foreign material has been destroyed, the plasma cells eventually die and antibodies stop being secreted. But the memory cells remain in the lymph nodes and the circulation. If a subsequent infection with the same pathogen occurs then some of these will turn into plasma cells and start producing antibodies. This is the basis of **natural active immunity** to diseases and underlies the practice of **immunisation** or **vaccination** which are methods of **artificial active immunity**.

Structure of an antibody

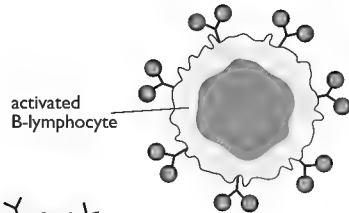


Humoral response to infection

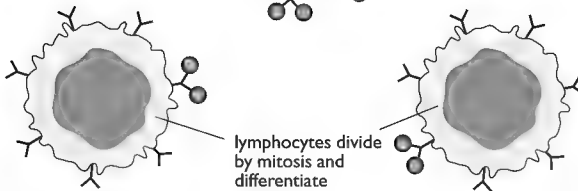
Immature B-lymphocyte



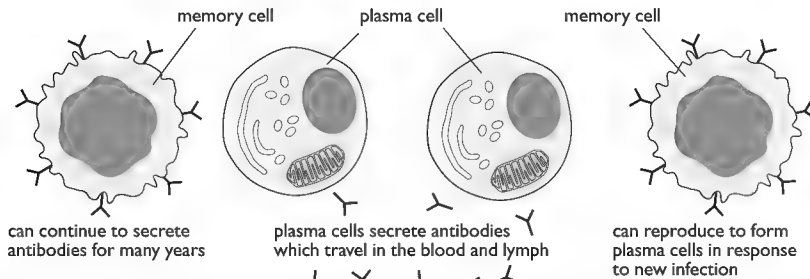
Activation



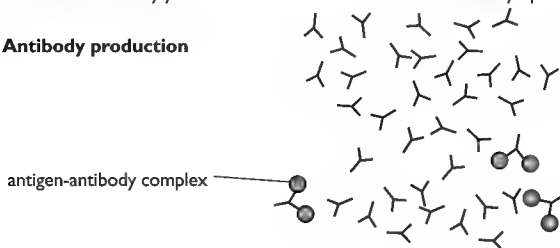
Division



Differentiation into plasma cells and memory cells



Antibody production



The cellular response of T- lymphocytes

One of the main ways in which the response of T-lymphocytes differs from that of B-lymphocytes is that T-lymphocytes do not produce antibodies but work directly on infected cells. As with B-lymphocytes, there are thousands of different types of T-lymphocyte which will each only recognise one specific antigen.

T-lymphocytes, like B-lymphocytes, have antigen receptors on their surface. However, unlike B-lymphocytes they cannot recognise antigens from pathogens on their own. They can only recognise them when they have been processed by human cells and presented on the surface of these cells. This rather complicated procedure relies on a group of special proteins called the **Major Histocompatibility Complex (MHC)** which are present on the surface of all human cells.

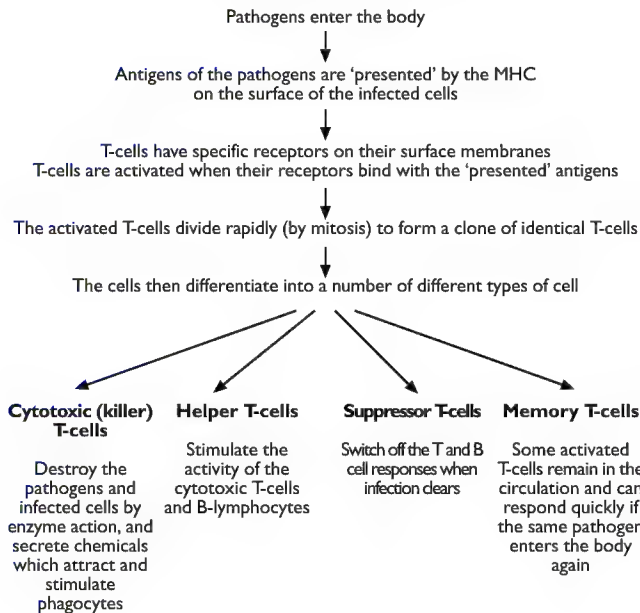
Normally the MHC allows the body's immune system to recognise its own cells and prevent their destruction. However, if a human cell becomes infected with a pathogen, then antigens from this pathogen interact with the MHC molecules and attach to them. They are now 'presented' on the surface of the cell. This alerts the body's immune system to the presence of the pathogen and allows T-lymphocytes to destroy the cell. The cell is known as an antigen presenting cell.

Antigens may also be presented by MHC molecules on the surface of B-lymphocytes and phagocytes. This is important as it allows the different parts of the immune system to cooperate with each other. Thus at the same time as producing antibodies, a B-lymphocyte can be presenting an antigen on its MHC and activating T-lymphocytes.

Sequence of events in cellular response

- ▼ Pathogen enters body.
- ▼ Antigens of pathogen which are presented by the MHC on a body cell come into contact with specific T-lymphocyte receptors which recognise them.
- ▼ T-lymphocyte binds to presented antigen on infected cell via antigen receptor.
- ▼ T-lymphocyte is activated and divides to form a clone of identical T-lymphocytes which enter the circulation and can attach to other infected cells.
- ▼ T-lymphocytes destroy the the pathogens and the cells to which they are bound. Some do this by releasing lytic enzymes into the infected human cells. Others release chemical messengers called cytokines which attract phagocytes to engulf the cells.
- ▼ Some T-lymphocytes then turn into memory cells which remain in circulation and in the lymph nodes in case of further infection.

Cellular Response to infection T-Lymphocytes (T-cells)



Helper and suppressor T-lymphocytes

The T-lymphocytes described above are called **cytotoxic T-lymphocytes**. There are also two other types of T-lymphocyte which work in different ways. **Helper T-lymphocytes** 'help' B lymphocytes and cytotoxic T-lymphocytes to function by releasing chemicals called cytokines and interleukins which stimulate the activity of the lymphocytes. They are only able to do this once they have been activated by binding to an antigen presented on an antigen presenting cell. It is these cells which are destroyed by the HIV virus (section 12.1). **Suppressor T-lymphocytes** are important in switching off the B and T-lymphocyte responses to a particular pathogen once the infection has been cleared.

◆ CHECKPOINT SUMMARY

- ◆ Antigens are defined as molecules, which, when foreign to an individual, stimulate the production of antibodies by B-lymphocytes.
- ◆ Antibodies are protein molecules known as immunoglobulins which are secreted by activated B-lymphocytes when a specific antigen is encountered.
- ◆ There are two main types of immune response: the Humoral Response which is associated with B-lymphocytes and involves the production of antibodies, and the Cellular or cell-mediated response which is associated with the T-lymphocytes.
- ◆ Binding of antigen to antigen receptor activates the B-lymphocyte and causes it to divide by mitosis producing a clone of identical B-lymphocytes.
- ◆ Most of the B-lymphocytes turn into plasma cells and the rest turn into memory cells.
- ◆ The plasma cells begin to secrete specific antibodies against the pathogen concerned.
- ◆ Once the foreign material has been destroyed, the plasma cells eventually die and antibodies stop being secreted. But the memory cells remain in the lymph nodes and the circulation.
- ◆ If a subsequent infection with the same pathogen occurs then some of these will turn into plasma cells and start producing antibodies. This is the basis of natural active immunity to diseases and underlies the practice of immunisation or vaccination which are methods of artificial active immunity.
- ◆ T-lymphocytes do not produce antibodies but work directly on infected cells.
- ◆ Antigens of pathogen which are presented on a body cell come into contact with specific T-lymphocyte receptors which recognise them.
- ◆ T-lymphocyte binds to presented antigen on infected cell via antigen receptor.
- ◆ T-lymphocyte is activated and divides to form a clone of identical T-lymphocytes which enter the circulation and can attach to other infected cells.
- ◆ T-lymphocytes destroy the pathogens inside the cells to which they are bound. Some do this by releasing lytic enzymes into the infected human cells. Others release chemical messengers called cytokines which attract phagocytes to engulf the cells.
- ◆ Some T-lymphocytes then turn into memory cells which remain in circulation and in the lymph nodes in case of further infection.

Primary and secondary immune responses

A primary immune response is the type of response which occurs when a pathogen is encountered by the body for the first time. A secondary response is that which occurs on a second or subsequent encounter with the same pathogen.

Both B-lymphocytes and T-lymphocytes can form memory cells after encountering a pathogen in the body on one occasion. The function of these is to allow the body to 'remember' pathogens it has encountered before and produce a much faster immune response to them the second time around.

T-lymphocyte memory

When a familiar antigen is encountered, T-lymphocyte memory cells will divide to form functional T-lymphocytes, enter the circulation and move to the area of infection. They are known to move to the area of the body where the specific infection occurred before. Some memory cells always remain in case of future infections.

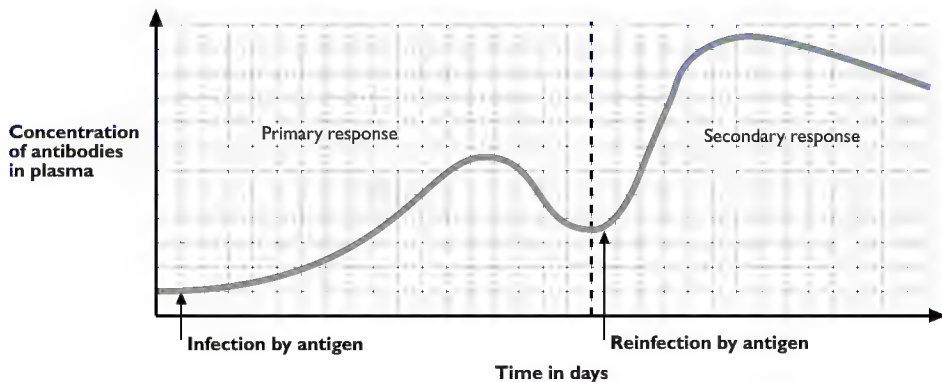
B-lymphocyte memory

B-lymphocyte memory cells produced during a primary encounter with a pathogen will divide and form new antibody producing plasma cells when they encounter their familiar pathogen again. Some memory cells remain in case of future infections.

Two main differences exist between the primary and secondary responses to a pathogen by B-lymphocytes.

- ▼ Antibodies are produced more rapidly in the secondary response.
- ▼ Larger amounts of antibodies are produced in the secondary response.

Primary and secondary immune response



The significance of memory cells

The presence of memory cells means that with some diseases such as mumps or chicken pox, one infection can make a person immune to that disease for the rest of their life. The pathogens causing these diseases will almost certainly get into their body again during that time, but the secondary immune response is so rapid that they will not become ill. With other infections, such as malaria, and influenza, the immunological memory is not so good and people can suffer many bouts of illness from the same type of pathogen. This is because some pathogens have a very high mutation rate in the genes coding for antigen proteins. The structure of the antigens changes slightly and the memory cells fail to recognise it.

Types of immunity: Active and Passive

The immunity to a disease which follows a natural infection with a pathogen (as described above) is known as **natural active immunity**. It involves a pathogen entering the body and allowing memory cells to be formed for future protection against the same disease. The person does not necessarily have to get ill from the infection for the immune system to work in this way.

In addition to this natural immunity there are two main ways in which immunity can be provided artificially. This is known as **immunisation** and includes vaccination (which produces **artificial active immunity**, often for life) and the provision of antibodies (**artificial passive immunity**) which provides immunity for a short period of time only.

Vaccination: artificial active immunity

Vaccination is a process by which immunity to a disease is achieved without experiencing a natural infection with that disease. It works on the same principle as natural active immunity, but instead of a person becoming infected with a pathogen via the normal routes, they are given a very small dose of antigens of the pathogen to which immunity is required via an injection or an oral vaccine. The different types of vaccine available are discussed below.

Following immunisation, B-lymphocytes are stimulated by antigens on the pathogen, producing plasma cells and hence antibodies, and memory cells. T-lymphocytes may also be stimulated and produce memory cells. These memory cells remain in the body and allow the person to respond quickly if they encounter the same pathogen again (during a natural infection), preventing them from becoming ill. This type of immunity is known as **artificial active immunity**, since the pathogen is introduced to the body in an artificial manner and the person does not usually get ill from the disease as they often do in natural active immunity. Some vaccines provide complete immunity after a single dose. Others require a second dose known as a 'booster' to allow a greater number of memory cells to form.

Vaccination is a safe and effective way of gaining immunity to diseases which might otherwise cause serious illness or death if caught naturally.

Types of vaccine

There are many different types of vaccine which have been developed to ensure that vaccinations are both effective and safe. It is usually unsafe to use a live natural pathogen, as even if given in small quantities, these may multiply rapidly and actually cause disease. Other options include:

- ▼ Vaccines containing a 'toxin' produced by the pathogen but not the whole pathogen. e.g. diphtheria.
- ▼ Vaccines containing dead (heat killed) pathogens, e.g. influenza. Even though the organism is dead its antigens can still be recognised and an immune response made against them.
- ▼ Vaccines containing a modified or weakened (attenuated) form of the pathogen. e.g. polio, *Rubella*, TB. In this case the microorganism is alive but has been modified in a way which prevents it from being harmful. It may have been treated with heat or chemicals to slow its rate of growth. Alternatively, naturally occurring mutant forms of the organism which have the same antigens, but do not cause disease may be used.
- ▼ Genetically engineered vaccines. Genetic engineering technology is allowing the development of new, safer and more effective vaccines. This is focussed on the identification of the genes of pathogens, and their removal, alteration or replacement.
- ▼ Genetically engineered vaccines may thus contain live pathogens which have been genetically modified by removing or 'switching off' potentially harmful genes. An example is the new cholera vaccine which is being developed from a cholera bacterium in which the two toxin genes have been removed. This should be much more effective than the current vaccine which uses dead cholera bacteria.
- ▼ In other instances the gene or genes coding for the antigenic proteins on the surface of a pathogen may be isolated, removed from the pathogen, and inserted into another form of bacterium or yeast. This can allow for the microbial production of vast amounts of antigen for use in vaccines. For example, the hepatitis B vaccine is a recombinant yeast vaccine. Hepatitis antigens are produced by yeast cells which contain a plasmid into which one of the viral genes responsible for antigen production has been inserted.

PASSIVE IMMUNITY

This is the process whereby ready made antibodies are given to a person to offer them rapid but short-term immunity to a disease. It is called passive because the person plays no role in actually producing the antibodies against that disease. Since memory cells are not given, such passive immunity will only last a few weeks or months, after which time the antibodies will be broken down by the body and stop working. There are two types of passive immunity.

Natural passive immunity occurs when antibodies are passed from mother to fetus via the placenta or from mother to baby via the colostrum (first milk). These antibodies protect the baby from diseases which it may encounter in the first few months of life before its own immune system starts functioning properly.

Artificial passive immunity occurs when antibodies produced in another organism are injected into a person to give them short term

protection against a specific disease. Antibodies may be given to a person who has already got the disease (such as someone who has got rabies from an infected dog bite) or to a person who is at risk of a catching a disease such as tetanus. In the past, such antibodies could only be obtained from the blood of other animals who had been deliberately infected with the pathogen.

Now some of the antibodies required for passive immunisation are produced as **monoclonal antibodies** in the laboratory. These are antibodies produced by a single clone of identical B-lymphocytes (plasma cells). They are produced by cells known as **hybridomas** which are created in the laboratory by fusing a plasma cell of the desired type (one that produces antibodies against a specific pathogen) with a cancer cell (myeloma). The hybridomas have the ability to constantly divide, like a cancer cell, and to produce antibodies like a plasma cell. The monoclonal antibodies produced have a variety of uses in medicine, research and diagnostic tests. (section 12.9).

◆ CHECKPOINT SUMMARY

- ◆ Passive immunity occurs when the individual plays no role in actually producing the antibodies against that disease. Since no memory cells are formed, such passive immunity will only last a few weeks or months, after which time the antibodies will be broken down by the body and stop working.
- ◆ Natural passive immunity occurs when antibodies are passed from mother to fetus via the placenta or from mother to baby via the colostrum (first milk). These antibodies protect the baby from diseases which it may encounter in the first few months of life before its own immune system starts functioning properly.
- ◆ Artificial passive immunity occurs when antibodies produced in another organism are injected into a person to give them short term protection against a specific disease.
- ◆ Antibodies required for passive immunisation can be produced as monoclonal antibodies in the laboratory.
- ◆ The immunity to a disease which follows a natural infection with a pathogen is known as natural active immunity.
- ◆ Immunisation includes vaccination (which produces artificial active immunity, often for life) and the provision of antibodies (artificial passive immunity) which provides immunity for a short period of time only.
- ◆ Vaccination is a process by which immunity to a disease is achieved without experiencing a natural infection with that disease. A very small dose of antigens of the pathogen to which immunity is required is given via an injection or an oral vaccine.
- ◆ Vaccines containing a 'toxin' produced by the pathogen but not the whole pathogen. e.g. diphtheria.
- ◆ Vaccines containing dead (heat killed) pathogens. e.g. influenza. Even though the organism is dead its antigens can still be recognised and an immune response made against them.
- ◆ Vaccines containing a modified or weakened (attenuated) form of the pathogen. e.g. polio, Rubella, TB.
- ◆ Genetically engineered vaccines may contain live pathogens which have been genetically modified or attenuated by removing or 'switching off' potentially harmful genes. Or the gene or genes coding for the antigenic proteins on the surface of a pathogen may be removed from the pathogen, and inserted into another form of bacterium or yeast. This can allow for the microbial production of vast amounts of antigen for use in vaccines.

3.4

Cell division

Content

Genetic information is passed from cell to cell during division

Mitosis

- ▼ The process of mitosis emphasising the behaviour of chromosomes, the role of the spindle and the genetic identity of the products.
- ▼ The use of appropriate staining techniques in the study of mitosis in suitable plant material (see practical work).

The cell cycle

- ▼ Mitosis and the cell cycle.
- ▼ The relationship between DNA replication and the events of the cell cycle.

Meiosis

- ▼ The importance of meiosis in halving the chromosome number in gametes so that, after fertilisation, the diploid chromosome number is restored in the resulting zygote.

MITOSIS

New cells (daughter cells) are formed by cell division. Cell division is initiated by the nucleus, and nuclear division is the first visible sign of the process of cell division. Mitosis is the commonest form of nuclear division, and results in two genetically identical daughter nuclei being formed. Mitosis occurs in the cell division seen in growth, repair, the replacement of cells which have a limited life span like our red blood cells and skin cells, and in asexual reproduction in many plants and lower animals. In the case of single celled organisms this type of asexual reproduction is called binary fission. The development of a multicellular organism from a fertilised egg by mitosis, means that all the cells of the body are genetically identical to the fertilised egg. The development of an organism from the fertilised egg is the story of the controlled expression of this genetic information, with only a small fraction of the genes being expressed in cells as they differentiate to form tissues specialised for specific (and limited) functions. In mature cells most of the DNA is inactive all of the time.

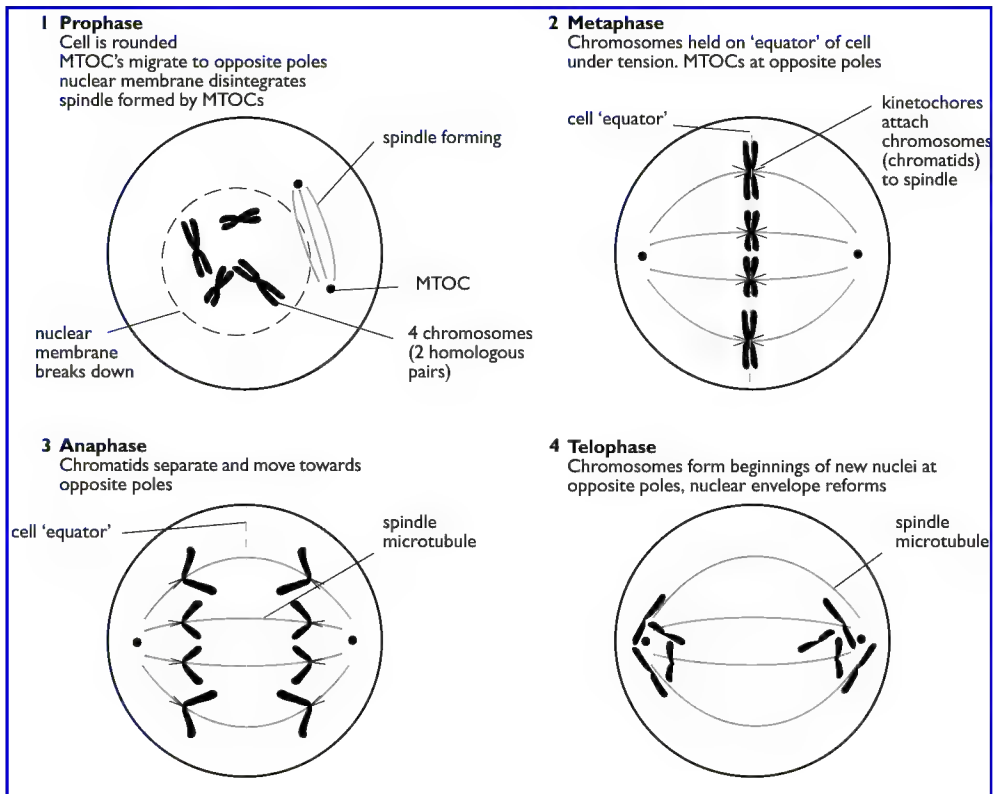
Asexual reproduction does not involve the fertilisation of gametes, and as a result of mitosis produces offspring which are genetically identical to each other (clones). Many multicellular organisms, especially plants can reproduce their entire bodies in this way, either from specialised structures, or single celled spores.

The production of genetically identical cells in the growth and development of multicellular organisms allows certain cells to retain the ability to develop into any other type if needed as a result of some damage. For example, stem cells in mammals can divide to form any of the different types of blood cell. In the asexual reproduction of organisms mitosis allows for the rapid exploitation of optimal conditions, where any variation would be disadvantageous.

The main stages of mitosis

Chromosomes only form and become visible (when stained and viewed under the light microscope) when the nucleus of a cell is just about to divide. This is because only at this time is the DNA/histone mixture wound up (condensed) as tightly as it can be. By this stage the DNA has also copied itself (replicated) so each chromosome appears as a double structure, joined at one point (the centromere). Whilst they remain joined in this way the two daughter chromosomes are referred to as chromatids.

Nuclear division (mitosis) occurs in four main stages, namely prophase, metaphase, anaphase and telophase.



Prophase is the first phase of mitosis, and as it begins, the DNA has already replicated, all protein synthesis has stopped, and each chromosome is condensed into the characteristic double chromatid form. At the same time the cell rounds up, the nuclear membrane breaks down into small fragments, and a spindle of microtubules forms between two microtubule organising centres (MTOCs) at opposite poles of the cell.

Metaphase is defined as when the chromosomes become attached to the spindle by structures called centromeres (kinetochores). Kinetochores consist of microtubules and 'motor' proteins which utilise ATP energy to pull on the spindle. The effect of both sets of kinetochores pulling in opposite directions is that the chromosomes line up along the middle (equator) of the spindle.

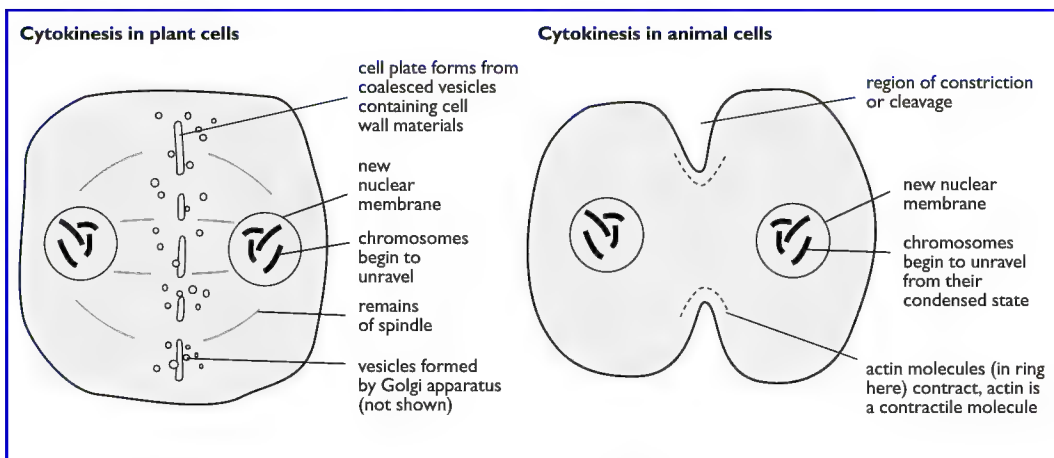
Anaphase occurs when quite suddenly, and all at once, the kinetochores and chromatids of each double chromosome separate, with each chromatid (now daughter chromosome) being pulled under tension in opposite directions towards the poles of the cell by the kinetochores, with their arms trailing in the characteristic 'V' shape.

Telophase is the stage when the two new, identical sets of chromosomes reach the opposite poles and a new nuclear envelope forms to surround each set.

Nuclear division by mitosis is followed by division of the cytoplasm into two equal parts. This process is called **cytokinesis** and it occurs differently in animals and plants.

In animal cells the spindle disintegrates during telophase and the microtubule components reassemble into a new cytoskeleton. The equator of the cell is constricted by a ring of contractile proteins (actin) in the process of cleavage, to create two roughly equal cells.

In plant cells the spindle lingers a little longer and seems to act as a guide to bring Golgi apparatus and associated secretory vesicles to the equator. The vesicles deposit their contents, which are the building constituents of a new cell wall, on the equator to form a plate of deposited material (the cell plate). Some of the vesicles remain intact and make connecting channels through the new cell wall (plasmodesmata).



THE CELL CYCLE

Interphase refers to the greater part of the life of a cell when there is no apparent activity. The period of mitosis and cytokinesis occupy only a fraction of the entire life of a cell. Together, interphase, mitosis and cytokinesis make up the **cell cycle**. The term interphase should not be interpreted to mean that the cell is resting. Interphase can itself be divided into three distinct periods called G1, S and G2. ('G' refers to the 'gaps' between DNA replication and mitosis.)

G1 is by far the longest period of the cell cycle. During G1, the cell machinery carries out protein synthesis, and growth occurs. All the cells of the body have identical sets of genes in their nuclei but the cells develop in different ways to perform different functions. This development process is called cell differentiation in which different genes are switched on and off, so that only one small part of the genetic code is active in each cell.

After a period of growth and differentiation, the length of which depends upon a variety of internal and external factors, protein synthesis is suspended, and the DNA set replicates itself. This is the **S** phase. DNA replication is followed by another 'gap' period, **G2**, before mitosis starts, in which the cytoskeleton of the cell breaks down and the microtubule components begin to reassemble into spindle parts. Without a cytoskeleton, the cell assumes a more spherical shape (see prophase).

The timing of events within the cell cycle is extremely variable. In ideal conditions, where food and oxygen are in plentiful supply and temperature and pH values are optimal, bacterial cells can complete the cycle to produce a new generation every 20 minutes. Other cells, for example muscle cells, never complete the cycle, a condition called terminal differentiation.

It has become clear that the onset of the S (DNA replication) phase and the M (mitotic) phase are determined by genes which are very similar in all organisms. These genes direct the synthesis of enzymes which in turn activate the enzymes initiating S and M phases.

The rate of cell division can be controlled by naturally occurring plant substances which affect the process of nuclear spindle formation. These include vincristine and vinblastine (from the periwinkle plant *Catharanthus rosea*). Both of these substances are used to control cancers such as leukemia and lymphoma. They work, at a molecular level, by binding on to the spindle microtubules, preventing spindle assembly.

The plant substance, taxol, from the Pacific Yew (*Taxus brevifolia*) has almost the reverse effect on spindle formation, causing an over production of microtubules which effectively stops cell division. Taxol has been used to treat ovarian cancers. As more is known about the factors controlling the cell cycle, new possibilities will open up for drug therapy which can be more specifically targeted. Work in this field will be aided by the research which has discovered the sequence of bases in the DNA of the human chromosomes (the Human Genome Project) as it reveals more and more about the predisposition of individuals with particular gene combinations to cancer.

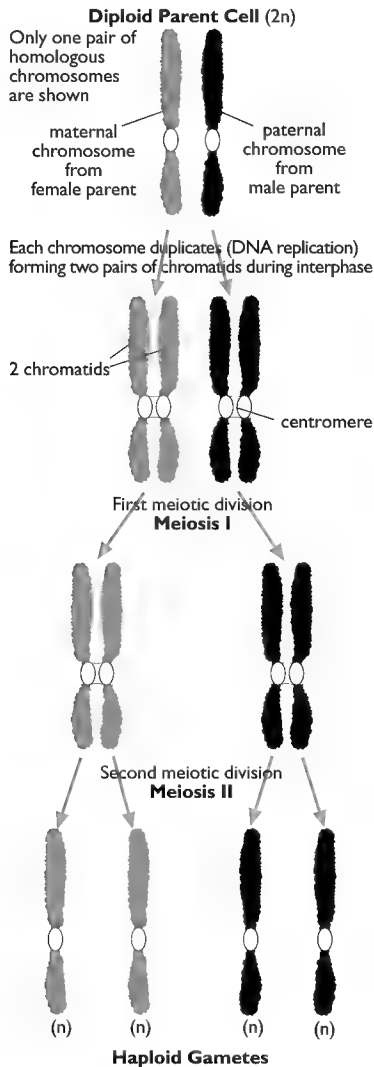
◆ CHECKPOINT SUMMARY

- ◆ Mitosis is nuclear division in which the daughter nuclei have the same chromosome number as the parent nucleus, typically diploid nuclei producing diploid daughter nuclei.
- ◆ The daughter nuclei are therefore genetically identical to the nucleus of the cell that produced them (except for any mutations that may occur).
- ◆ Mitotic cell division is seen in growth, repair and asexual reproduction.
- ◆ As a result of mitosis in the growth of an organism from the fertilised egg all cells therefore are genetically identical (totipotent).
- ◆ Similarly, offspring of asexual reproduction lack genetic variation.
- ◆ The production of genetically identical cells retains the full set of genetic information needed for the replacement of any cells in repair processes, and in the asexual reproduction of organisms it allows for the rapid exploitation of optimum conditions where any variation would be disadvantageous.
- ◆ The DNA replicates in interphase so that in the first stage of mitosis (prophase) the chromosomes appear as double structures of two chromatids (daughter chromosomes) joined at the centromere.
- ◆ The nuclear spindle apparatus(NSA) forms from the dissolution of the nuclear membrane and the centrioles replicate and migrate to either pole of the NSA.
- ◆ The centromeres align on the equator of the NSA (metaphase).
- ◆ The centromeres divide and the chromatids are moved to the opposite poles (anaphase).
- ◆ Two new daughter nuclei are formed (telophase).
- ◆ The cytoplasm is divided between two daughter cells by cleavage in animal cells and the laying down of new cell walls in bacteria and plants
- ◆ The formation of two new cells completes the cell cycle.

MEIOSIS

Meiosis is a special type of cell division called a **reduction division**. Meiosis occurs during the production of sex cells (gametes) in animals, and in spore formation which precedes gamete production in plants. Meiosis results in the number of chromosomes being halved from the normal **diploid** to the **haploid** number. The mechanisms of cell division are very similar to that already described for mitosis, but meiosis involves two divisions, referred to as the first and second divisions (meiosis I and meiosis II), and the result of meiosis is four cells (gametes or spores) each containing half the original number of chromosomes.

Meiosis involves certain genetic 'mixing' events, and these, along with the random fusion of the gametes in fertilisation to form a diploid zygote, result in genetic variation in the offspring which may be of adaptive advantage.



◆ CHECKPOINT SUMMARY

- ◆ Each gamete of a sexually reproducing organism contains one set of chromosomes (haploid).
- ◆ When two such gametes fuse in fertilisation they produce a zygote with two sets of chromosomes (diploid).
- ◆ Chromosomes in a diploid nucleus thus occur in pairs, each member of a pair carrying the same genes in the same relative positions (homologous chromosomes).
- ◆ Slight variations (alleles) of a gene at a particular locus can arise by mutation, so that an organism can either have an identical pair of genes at a locus (homozygous) or a pair that differs slightly (heterozygous).
- ◆ Pairs of sex determining chromosomes are only partially homologous e.g. the human Y chromosome has a short non-homologous arm which carries the male determining genes.
- ◆ The production of haploid gametes from a diploid cell requires a reduction division (meiosis) in which the chromosome number is halved from diploid to haploid.
- ◆ The production of haploid gametes ensures the maintenance of the diploid chromosome number in subsequent generations.

3.5

DNA and protein synthesis

Contents

DNA as genetic material

- ▼ The structure of DNA, mRNA and tRNA in terms of nucleotides, base pairing and hydrogen bonding.
- ▼ Evidence that DNA is the genetic material

Nucleic acids and DNA replication

- ▼ The roles of nucleic acids in coding information, protein synthesis and the replication of DNA
- ▼ The semi-conservative replication of DNA

Protein synthesis

- ▼ The genetic code as a non-overlapping, degenerate code. Introns as non-coding DNA.
- ▼ The mechanism of protein synthesis involving the roles of mRNA, tRNA and the ribosomes.
- ▼ DNA determines the structure of enzymes which determine the phenotype of an organism, illustrated by reference to cystic fibrosis and phenylketonuria

DNA AS GENETIC MATERIAL

The structure of DNA and RNA

DNA (deoxyribo(se)nucleic acid), is so named because it is an acid and it is found in the nucleus. It is a polymer made up of repeating sub-units, in this case, nucleotides linking up to form a polynucleotide. What makes DNA so important and so unique is that it contains within its structure the genetic code, and it can produce copies of itself (replicate).

Evidence that DNA is the genetic material

The nucleus of cells contains mostly DNA and protein. For many years it was thought that protein was the hereditary material, but when it became possible to extract and measure the quantity of DNA, it was found that the DNA content doubled in cells just before dividing. It was also found that, in the formation of sex cells, the amount of DNA halved. Both pieces of evidence underline its central role in inheritance. Now it has become possible to analyse the molecular structure of DNA, to unravel the chemical code it contains, and to transfer pieces of this code between one organism and another in the process known as genetic engineering.

DNA is a polynucleotide made up of repeating nucleotide units. A **nucleotide** consists of three main parts: a pentose (5 carbon) sugar,

joined to a phosphate group, and a nitrogen-containing organic base. There are four different types of nucleotide in DNA each with a different organic base. The bases are **adenine** (A) and **guanine** (G) which belong to a group of chemicals called **purines**, and **cytosine** (C) and **thymine** (T) which belong to group called **pyrimidines**. Chains of nucleotides link up by condensation reactions, with the sugar and phosphate molecules forming the 'spine' of each strand of the molecule, with the bases to one side.

DNA consists of two such chains or strands linked by **hydrogen bonds** which form between pairs of bases. As the hydrogen bonds form, so the two strands of the DNA molecule twist into the spiral structure known as the double helix. Hydrogen bonds form only between specific pairs, namely A with T and C with G. The strands are therefore **complementary** to each other. The sequence along one strand determines the sequence of the other (complementary) strand. The understanding of the structure of the DNA double helix was one of the most significant breakthroughs in Biology.

RNA (Ribo(se)nucleic acid), like DNA, is a polymer made up of repeating nucleotides. It differs from DNA in the following three ways.

RNA is single stranded, not double stranded.

The pentose sugar in RNA is ribose, not deoxyribose (molecules of ribose contain an additional oxygen atom).

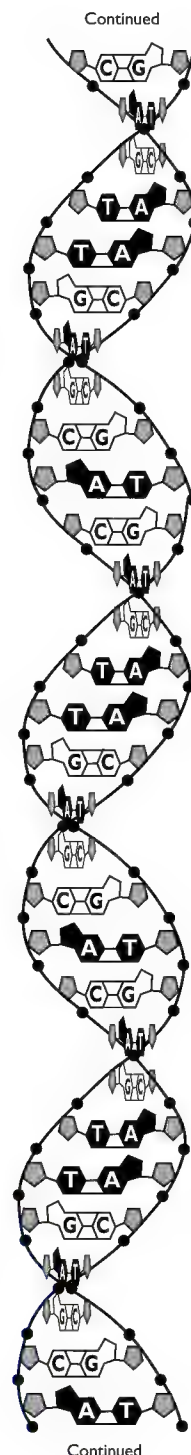
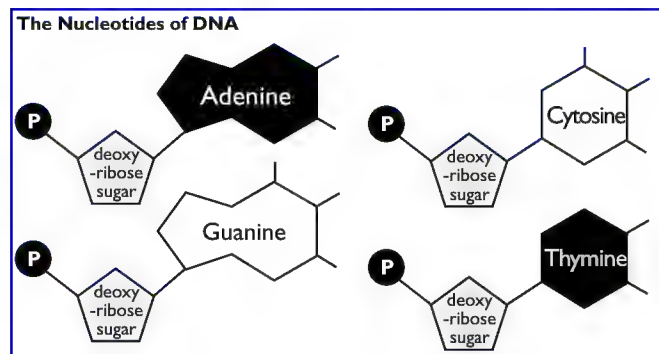
The pyrimidine base Uracil (U) is found instead of Thymine (T).

Living cells produce three different types of RNA in their nuclei, each designed to carry out a specific function in protein synthesis.

Messenger RNA (mRNA) consists of a single strand, between 75 and 3000 nucleotides long, produced in the nucleus as an exact copy of a selected section of the DNA code. The mRNA molecules travel out of the nucleus to the ribosomes where proteins are synthesised.

Transfer RNA (tRNA) is a shorter length of RNA, between 75 and 90 nucleotides long, twisted into a characteristic shape which allows one end to attach to a specific amino acid, and the other to expose a triplet of bases called the anti-codon which attaches to mRNA at the site of protein synthesis. tRNA molecules bring amino acids to their correct position as the proteins are being assembled. Each type of amino acid is carried by a different type of tRNA molecule with a specific anti-codon.

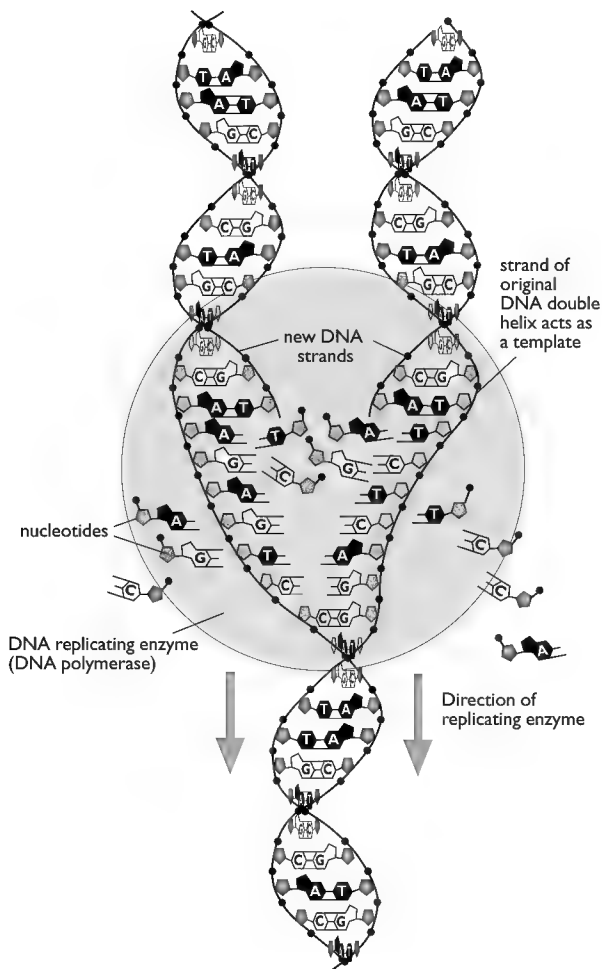
Ribosomal RNA (rRNA) consists of more than 1000 nucleotides and is found in ribosomes which are composed of an approximately equal mixture of proteins and rRNA. Ribosomal RNA is manufactured within the nucleolus.



Replication of DNA

In the process of DNA replication the original strands separate, and each acts as a pattern (template) for the formation of another new strand of DNA. Each of the daughter DNA molecules retains (conserves) one of the original strands and has one new one. For this reason, the process of replication is said to be **semi-conservative**. The process requires a supply of the four different nucleotides, energy in the form of ATP, and an enzyme called DNA polymerase, which is required to catalyse the condensation reactions by which free nucleotides form a copy of the template strand.

Replicating DNA enzyme



◆ CHECKPOINT SUMMARY

- ◆ The nucleus of cells contains mostly DNA and protein.
- ◆ The DNA content doubles in cells just prior to dividing.
- ◆ It was also found that, in the formation of gametes (sperms and eggs), the amount of DNA halved. This evidence underline its central role in inheritance.
- ◆ Now it has become possible to analyse the molecular structure of DNA, to unravel the chemical code it contains, and to transfer pieces of this code between one organism and another in the process known as genetic engineering.
- ◆ DNA and RNA are long chain polynucleotide molecules with repeating sub-units of nucleotides.
- ◆ Nucleotides consist of a 5 carbon sugar, a phosphate and an organic base.
- ◆ In DNA the sugar is deoxyribose and the organic base can be one of adenine, thymine, cytosine and guanine.
- ◆ In RNA the sugar is ribose and the organic base can be one of adenine, uracil, cytosine and guanine.
- ◆ The DNA molecule is a double stranded helix with the two strands held together by hydrogen bonds between complementary base pairs adenine-thymine and cytosine-guanine.
- ◆ RNA is a single stranded helix with base pairs of adenine-uracil and cytosine-guanine.
- ◆ There are three types of RNA - messenger, transfer and ribosomal.
- ◆ DNA carries out semi-conservative replication in which each strand of the original molecule acts as a template for the construction of a new one, so that each new double stranded molecule of DNA consists of one original strand and a new complementary strand.

Experimental evidence for the semi-conservative mechanism of DNA replication includes experiments in which the mass of the DNA content of *E. coli*, grown on different nutrient media was determined and compared. One batch of microorganisms was cultured on a medium containing a 'heavy' isotope of nitrogen (^{15}N), the DNA was extracted and compared with that of organisms cultured on a medium containing normal 'light' nitrogen (^{14}N). The *E. coli* cells grown on 'heavy' nitrogen were then transferred to a new medium containing only light nitrogen and allowed to divide once to produce a new generation. The DNA of these daughter cells was found to be halfway between the 'heavy' and 'light' values, indicating that half the DNA was old and half was new (semi-conservative replication).

The genetic code

The DNA base sequence forms a four letter language which, as you have seen, can be copied every time a cell divides. This DNA base sequence determines the sequence of amino acids in a polypeptide chain (the primary structure of proteins). A sequence of bases which codes for one polypeptide is known as a gene. Each gene codes for a different polypeptide chain. The primary structure of a polypeptide is the basis of the particular structural shape and characteristic of the final protein, e.g. enzymes, structural materials and membrane proteins. The base code determines the structure, functions and individuality of every organism.

Remember that up to twenty different types of amino acids may occur in a single protein. The DNA code has a four letter base language (A,G,C,T). A triplet of bases (sequence of three in a row) codes for each amino acid, for example 'CGC' codes for the amino acid alanine (Ala) whilst 'GCT' codes for arginine (Arg), so the code

Amino acid	Abbr.	mRNA codons	Essential
Alanine	Ala	GCA, GCC, GCG, GCU	
Arginine	Arg	AGA, AGG, CGA, CGC, CGG, CGU	infants only
Asparagine	Asn	AAC, AAU	
Aspartic acid	Asp	GAC, GAU	
Cysteine	Cys	UGC, UGU	
Glutamic acid	Glu	GAA, GAG	
Glutamine	Gln	CAA, CAG	
Glycine	Gly	GGA, GGC, GGG, GGU	
Histidine	His	CAC, CAU	infants only
Isoleucine	Ile	AUA, AUC, AUU	E
Leucine	Leu	CUA, CUC, CUG, CUU, UUA, UUG	E
Lysine	Lys	AAA, AAG	E
Methionine	Met	AUG (Start codon)	E
Phenylalanine	Phe	UUC, UUU	E
Proline	Pro	CCA, CCC, CCG, CCU	
Serine	Ser	AGC, AGU, UCA, UCC, UCG, UCU	
Threonine	Thr	ACA, ACC, ACG, ACU	E
Tryptophan	Trp	UGG	E
Tyrosine	Tyr	UAC, UAU	
Valine	Val	GUA, GUC, GUG, GUU	E
STOP CODONS		UAA, UAG, UGA	

'CGC-GCT-CGC' would be translated as 'Ala-Arg-Ala' in a polypeptide. Note that the code is **non-overlapping**, i.e. it must be read **CGC-GCT-CGC**, not **CGC-GCG-CGC-GCT-CTC** etc.

Sixty four different triplet codes (codons) are possible with this four letter base language, more than sufficient to code for the twenty different types of amino acids. The term **degenerate** is applied to the genetic code because there are more codons available than can be used to code for amino acids. In fact most amino acids are coded for by more than one codon each. The process by which this code is used in protein synthesis, is described below.

Protein Synthesis

Protein synthesis involves all three of the RNA types described above working in combination, and is achieved in two stages. As the DNA cannot leave the nucleus, first the code has to be read and copied (transcribed) into mRNA, in a process called **transcription**. The mRNA moves out of the nucleus and associates with the ribosomes, where the code (now embedded in the mRNA) is used in the synthesis of a polypeptide in the process of translation.

Transcription begins as an enzyme, **RNA polymerase**, attaches to the **'Start' codon** of a gene. The DNA base sequence of this 'Start' codon is always 'TAC'. As RNA polymerase attaches to the DNA, the hydrogen bonds binding the two strands of the DNA double helix break apart to expose the sequence of triplet base codons on both strands. The enzyme then travels rapidly down the length of the gene like a zip fastener unzipping the DNA. As it does so, a molecule of mRNA is formed copying one of the DNA strands. Only one of the two DNA strands (the template strand) is copied into mRNA. The mRNA is therefore an exact replica (save that 'U' replaces 'T') of the other strand, consequently referred to as the **copy strand**. When the enzyme reaches the 'Stop' codon of the gene, transcription ends, the enzyme is free to start the process again, and mRNA is released out through a pore in the nuclear membrane to the ribosomes where the next stage occurs.

A complicating feature of the process of transcription in eukaryotic cells (cells with a nucleus) is that the length of DNA to be copied into mRNA (the gene) has sections of **'junk code'** which do not code for any of the required amino acids in a polypeptide chain. The 'junk' sequences are called **introns** (because they stay within the nucleus) and the parts to be transcribed and translated are called **exons**. The whole length is transcribed into mRNA, but before it is released from the nucleus, the introns are cut out with the assistance of enzymes, leaving a single, 'unpolluted', strand.

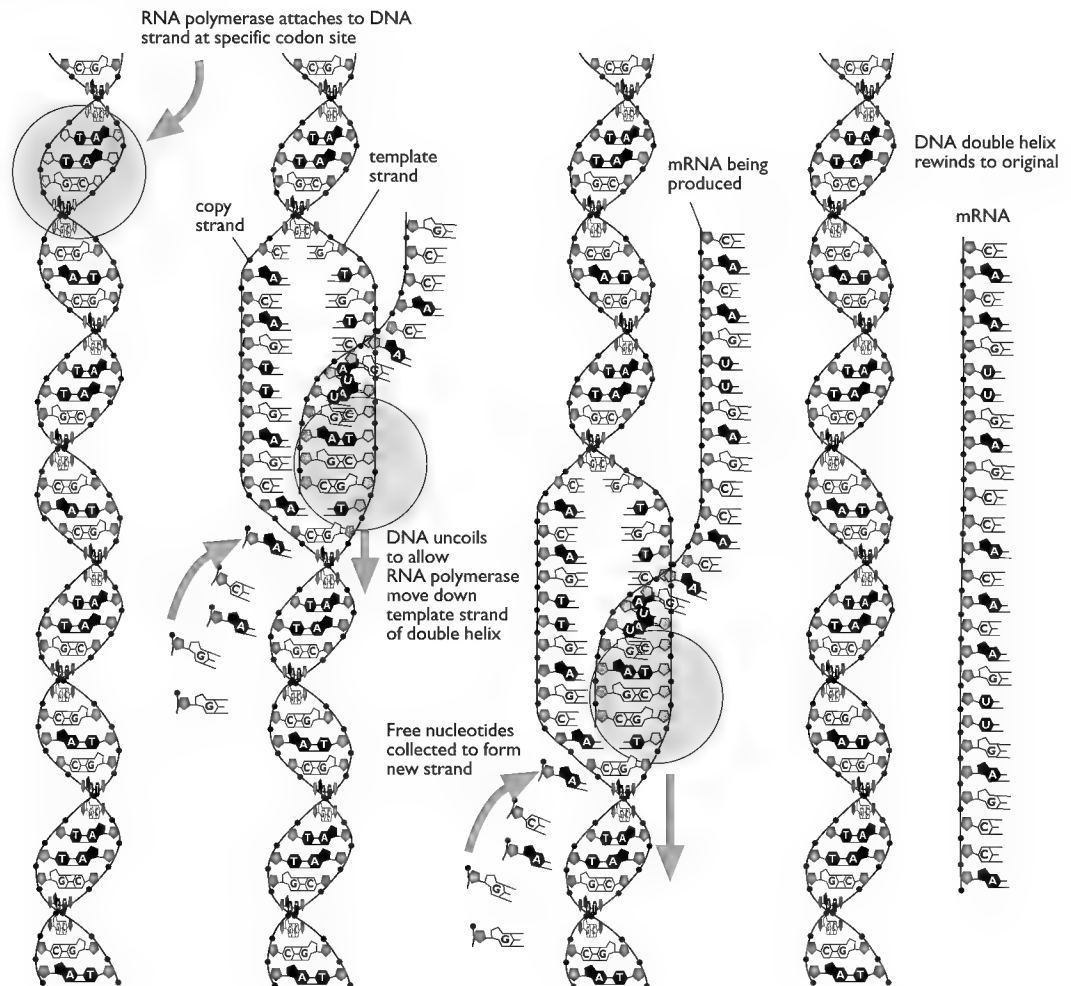
Translation occurs at ribosomes which are made up of two sub-units, one larger than the other, which come together only for the purpose of translating a length of mRNA. The mRNA binds to the small sub-unit at its 'Start' codon (AUG). It is then joined by the large sub-unit along with a molecule of tRNA which has the anti-codon 'UAC' and which carries the amino acid methionine. As the three different RNA types come together, the interface between the small and large sub-unit of the ribosome forms two binding sites allowing two triplet codons be lined up side by side. The process of translation can then proceed.

A second tRNA now occupies a position on the mRNA alongside the first and, as it does so, a peptide bond is formed between the two

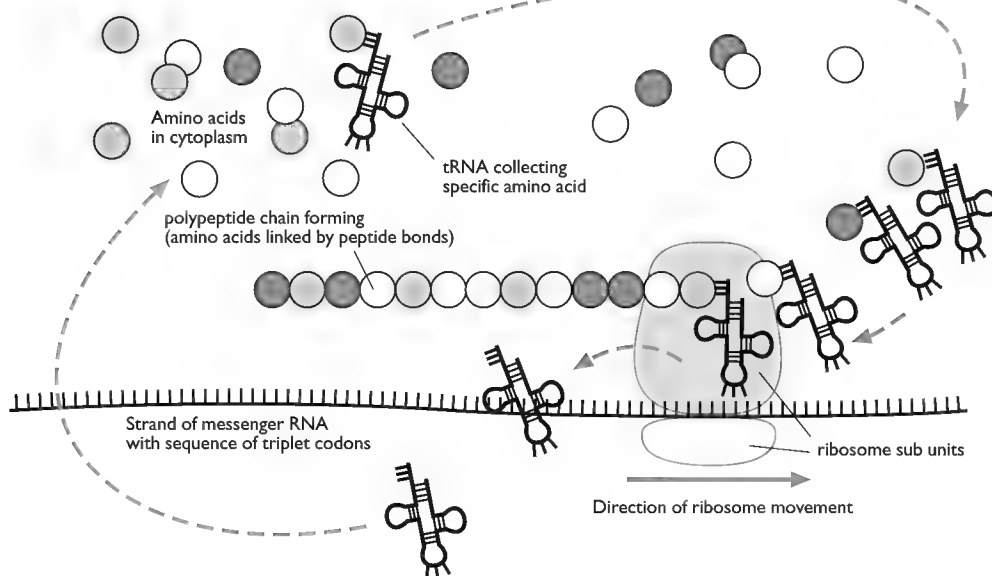
amino acids. The ribosome moves along the length of the mRNA, and once a tRNA molecule has delivered the appropriate amino acid it is released to repeat the process. New tRNA molecules with their amino acids come to occupy the ribosomal coding sites, and the polypeptide chain grows ever longer. When the ribosome reaches the '**Stop**' codon (UAG) on the mRNA, it splits into its separate sub-units releasing the mRNA and the now complete polypeptide. A number of ribosomes move along the mRNA at the same time so that many polypeptide molecules are formed simultaneously. It is calculated that, in the average mammalian cell, more than a million new peptide bonds are being formed every second.

Generally mRNA molecules only have a short existence, sometimes just a few minutes, so that if continuous supplies of a particular protein are required the above processes must be repeated.

Transcription



Translation of mRNA code to build a polypeptide



◆ CHECKPOINT SUMMARY

- ◆ The sequence of bases (and hence nucleotides) on one strand of the DNA molecule carries the genetic code for the synthesis of proteins and hence ultimately living cells.
- ◆ Three bases (a triplet codon) code for one amino acid.
- ◆ There are 64 possible combinations of any three from four bases, but only 20 amino acids to be coded for.
- ◆ Amino acids are coded for by more than one triplet codon and the code is said to be 'redundant' or 'degenerate'.
- ◆ A gene is a sequence of nucleotides of a DNA molecule which codes for a polypeptide.
- ◆ A gene for a polypeptide has a specific 'start' codon which thus sets the order of all the subsequent triplet codons
- ◆ Thus the code is non-overlapping i.e. a base can only be 'used' in one triplet codon.
- ◆ Only a fraction of the nuclear DNA contains genes coding for proteins, the rest consists of so-called 'junk' DNA the function of which is not understood
- ◆ Nuclear DNA with the genetic code never leaves the nucleus in eukaryotic cells.
- ◆ The genetic code for a polypeptide (a gene) is copied (transcribed) into mRNA which leaves the nucleus and associates with the ribosomes.
- ◆ The ribosomes 'read' the genetic message on the mRNA and tRNA deliver amino acids in the sequence determined by the code.
- ◆ There are 20 different types of tRNA, each specific to a particular amino acid.
- ◆ Each tRNA has a specific binding site at which the specific amino acid combines and an anti-codon which matches the specific codon for that particular amino acid.
- ◆ The amino acids combine by means of peptide bonds to give a polypeptide chain.
- ◆ The polypeptides subsequently form proteins.
- ◆ As enzymes are proteins their synthesis is controlled by DNA.

DNA control of enzyme synthesis

Since the base sequence of DNA in a gene controls the production of one specific polypeptide or protein, it follows that any change in this base sequence (a gene mutation) may lead to the production of a faulty protein. Even a substitution or deletion of a single base can alter the translation of the DNA code into a sequence of amino acids in a polypeptide chain. If the primary structure of a polypeptide is altered this can affect its secondary, tertiary and quaternary structures, with corresponding effects on its function. The presence of such a faulty protein may in turn affect the appearance and functioning (phenotype) of the organism as a whole and cause a genetic disease or disorder. Gene mutations which occur during the production of gametes are thus the cause of genetic diseases in the offspring.

Some genetic diseases are referred to as 'inborn errors of metabolism' because they affect one or more of the metabolic pathways involved in the normal functioning of the organism. They may, for example, include diseases where particular enzymes necessary for metabolic functions are faulty or absent, e.g. Phenylketonuria (PKU). In this example the pathway from faulty gene to abnormal phenotype is clear. Identification of the gene and protein product responsible for the disease can allow medical intervention at any point in the pathway from gene to disease.

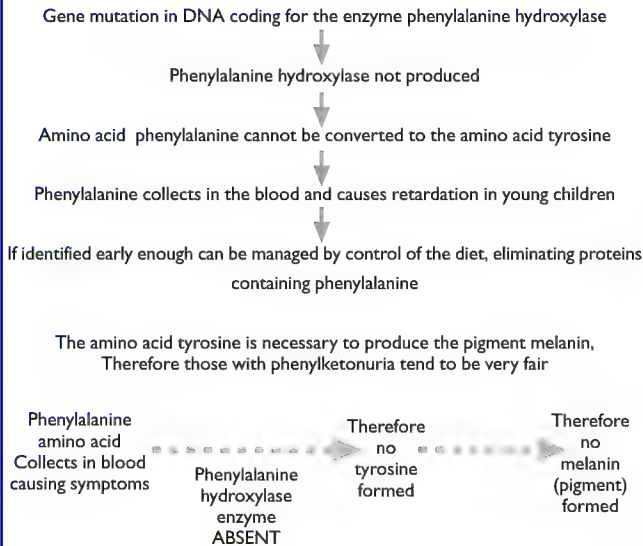
Phenylketonuria (PKU)

Most humans are able to convert some types of amino acids into other types of amino acid in the liver and thus produce all the proteins necessary for growth, repair and functioning of tissues. This explains why some amino acids are said to be essential (must be taken in the diet) whilst others are non-essential (do not need to be taken in the diet as they can be made from the essential amino acids in the body). Conversion of one amino acid to another (transamination) allows the body to make full use of ingested proteins and also prevents the build up of particular unused amino acids which could be dangerous.

In the disease phenylketonuria (PKU), however, a gene mutation in the DNA coding for the enzyme phenylalanine hydroxylase means that this enzyme is not produced in the liver. Without phenylalanine hydroxylase the body cannot convert the amino acid **phenylalanine** to the amino acid **tyrosine**. This results in an accumulation of phenylalanine in the body which severely affects many systems. Untreated sufferers may be both mentally and physically retarded.

PKU can be treated by modifying the diet to ensure that it lacks, or is very low in, the amino acid phenylalanine. The amino acid tyrosine may also be given. Under these circumstances individuals will not develop any signs of retardation. In the UK, all new-born babies are screened for PKU using a simple blood test which allows individuals with this genetic condition to be detected and treated before damage occurs. Treatment is only necessary in children, as adults seem to be unaffected by the high phenylalanine levels.

Phenylketonuria



SUMMARY OF PKU

- ▼ Gene mutation occurs in DNA coding for the enzyme phenylalanine hydroxylase
- ▼ Enzyme phenylalanine hydroxylase not produced
- ▼ Faulty metabolic pathway: amino acid phenylalanine not converted to tyrosine
- ▼ Phenylalanine accumulates with toxic effects
- ▼ Abnormal phenotype: Mental and physical retardation occurs

Cystic fibrosis

Cystic fibrosis is one of the most common autosomal recessive genetic diseases and affects about 1/2000 babies born in the UK. About 1:23 of the population are carriers of the cystic fibrosis allele. The disease which primarily affects the lungs, pancreas and liver is characterised by the production of thick, sticky mucus by epithelial cells in these regions. It usually causes death by early adulthood.

The disease is caused by a faulty protein known as **CFTR** (cystic fibrosis transmembrane regulator) which regulates transmembrane chloride ion channels in epithelial cells. A deletion of 3 bases in the DNA code of the CFTR gene means that one amino acid (phenylalanine) is missing from the CFTR protein which is produced. This change in the primary structure of the protein alters its secondary and tertiary structures, preventing it from responding to signals to open chloride channels in cell membranes. The overall effect of this is a build up of ions inside the cells which prevents water leaving by osmosis as it would do normally. The mucus released by cells is thicker than in normal individuals and sticks to cells causing inflammation and tissue damage, encouraging infections and preventing normal functioning of the organs concerned.

Although the disease is not fully understood, a number of treatments are available. There is, however, no cure. One experimental approach to the treatment of Cystic fibrosis in the lungs involves a form of gene therapy in which sufferers inhale normal CFTR genes incorporated into viruses or liposomes. Other treatments involve attempts to stimulate chloride ion secretion by the cells with the use of ATP.

SUMMARY OF CYSTIC FIBROSIS

- ▼ Deletion of bases occurs in DNA of CFTR gene
- ▼ Amino acid phenylalanine missing in CFTR protein
- ▼ Secondary and tertiary structure of CFTR disrupted
- ▼ CFTR protein fails to regulate opening of chloride ion channels in epithelial cells
- ▼ Chloride ions stay inside cells, as does water; and sodium ions are attracted in.
- ▼ Mucus produced is thick and sticky
- ▼ Mucus adheres to epithelial cells and causes disease symptoms

Cystic Fibrosis

Mutation causes the deletion of 3 bases in DNA so that one amino acid (phenylalanine) is not coded for in the CFTR protein (Cystic fibrosis transmembrane regulator)



Faulty CFTR protein cannot control the opening of chloride channels in the cell membrane



Results in production of thick sticky mucus, especially in lungs, pancreas and liver
Organs cannot function normally and infection becomes more likely

Treatment by Gene Therapy

CFTR gene identified on chromosome 7 in 1989

The normal gene for CFTR protein is inserted into a bacterial plasmid (DNA).
This is enclosed in a liposome (hollow lipid container)

The liposomes are introduced into the body of the sufferer using a nasal spray

The liposomes reach the lung epithelial cells and hopefully some of the normal CFTR genes are incorporated into the cells' DNA.
(The normal gene could also be introduced using a 'safe' virus)

◆ CHECKPOINT SUMMARY

- ◆ Some genes necessary for metabolic functions are faulty or absent, e.g. Phenylketonuria (PKU) and cystic fibrosis.
- ◆ In the disease phenylketonuria (PKU) a gene mutation in the DNA coding for the enzyme phenylalanine hydroxylase means that this enzyme is not produced in the liver.
- ◆ Without phenylalanine hydroxylase the body cannot convert the amino acid phenylalanine to the amino acid tyrosine.
- ◆ This results in an accumulation of phenylalanine in the body which severely affects many systems. Untreated sufferers may be both mentally and physically retarded.
- ◆ Cystic fibrosis primarily affects the lungs, pancreas and liver, and is characterised by the production of thick, sticky mucus by epithelial cells in these regions. It usually causes death by early adulthood.
- ◆ The disease is caused by a faulty protein known as CFTR (cystic fibrosis transmembrane regulator) which regulates transmembrane chloride ion channels in epithelial cells.
- ◆ A deletion of 3 bases in the DNA code of the CFTR gene means that one amino acid (phenylalanine) is missing from the CFTR protein which is produced.
- ◆ This change in the primary structure of the protein alters its secondary and tertiary structures.
- ◆ CFTR protein fails to regulate opening of chloride ion channels in epithelial cells
- ◆ Chloride ions stay inside cells, as does water. Sodium ions are attracted in.
- ◆ Mucus produced is thick and sticky
- ◆ Mucus adheres to epithelial cells and causes disease symptoms.

3.6

Gene technology

Contents

Recombinant DNA

- ▼ The production of recombinant DNA and its use in the production of human insulin and other proteins
 - isolation of the required gene
 - use of the enzymes: reverse transcriptase, restriction endonuclease and ligase
 - sticky ends: insertion of the gene into a vector and its subsequent introduction into host cells; plasmids and viruses as examples of vectors
 - use of genetic markers such as genes conferring antibiotic resistance to detect genetically modified organisms
 - multiplication of host cells

The moral and ethical issues associated with recombinant DNA technology

RECOMBINANT DNA

Imagine how simple life would be if there were only one type of computer and only one type of software. All the machines could then use the same language and all the information would be interchangeable. This is exactly how it is with the language of the genes. The synthesis of polypeptides from amino acids relies on exactly the same mRNA codons in every living organism so, for example, 'GCG' codes for the amino acid alanine and 'CGA' codes for arginine whether it occurs in a human, a honey bee or a mushroom. Now that genes can be identified and isolated, gene technology is providing a means for transferring them from one organism to another.

The terms 'genetic modification', 'genetic engineering' and 'gene technology' refer to the processes and techniques by which genes may be extracted from the DNA of one organism and inserted into the DNA of another organism (the **host** organism). In this way, the gene set (**genome**) of the host can be **transformed** (genetically modified). The modified DNA is called **recombinant DNA** because it has been re-combined to make a different set of codes.

Examples of genetically engineered products

Insulin is needed by people who are unable to produce sufficient quantities of the hormone and who would otherwise develop the disease **diabetes mellitus**. This is a condition, affecting 2% of the population, in which blood sugar rises to dangerous levels. Before gene technology, the only source of insulin was natural. It was extracted from the pancreases of pigs and cattle. This was not ideal because human insulin is slightly different in chemical structure,

and although the pig and cattle version works on glucose in a similar way, some people are allergic to it and the process is both costly and open to contamination by impurities.

In the early 1980s the gene for human insulin was transferred from human pancreas cells to *E. coli* bacteria. Bacterial colonies modified in this way were cultured for mass production in industrial fermenters providing a constant supply of insulin. Now most insulin is produced by transformed bakers' yeast cultures. Yeast has various advantages over bacteria. It does not produce any toxic by-products, it can be grown on simple nutrient media, and it can secrete the finished product directly into the culture medium making the harvesting process easier.

Factor VIII is a vital blood clotting factor lacking in people suffering from **haemophilia**. Natural sources are both expensive and liable to contamination but now the gene for factor VIII has been engineered into yeast cells.

The food industry uses more and more genetically engineered products. One of the first to be developed on a commercial scale was the enzyme **chymosin** (rennilase) which is used to coagulate milk for the production of cheese. Chymosin occurs naturally in the stomach of young mammals, where it is often referred to as rennin. The cheese making industry relied on extracts from calves' stomachs until the advent of gene technology. Now chymosin can be obtained from modified yeast cultures grown in fermenters.

The techniques of Genetic Manipulation

Gene technology relies on the fact that the genetic language is universal; the 'software' (genes) can be used in any living organism. The difficulty lies in the 'cutting and pasting' of genetic information from one organism to another, and you must first become familiar with three enzymes which are crucial to this process.

Reverse Transcriptase

Reverse transcriptase is an enzyme which makes a single strand of DNA from an RNA template. From this strand of DNA, a complementary strand can be assembled with the aid of DNA polymerase. Reverse transcriptase enzyme was identified and isolated from **retroviruses**, a group of viruses which includes the HIV virus. The genetic material of retroviruses is RNA (not DNA), and when retroviruses invade cells their RNA uses reverse transcriptase to make a piece of DNA. This becomes incorporated into the host cell's own DNA and directs the production of new viruses (an example of natural genetic modification of the host cell).

Reverse transcriptase is used in gene technology to make DNA from mRNA. Remember that mRNA is a copy of a single gene and that mRNA of a working gene may be found in the cytoplasm of a cell. It is possible to isolate a gene in the form of mRNA and then to multiply it using a combination of reverse transcriptase and DNA polymerase.

Restriction endonuclease enzymes

Another vital tool for gene technologists are enzymes called restriction endonucleases (restriction enzymes) which act like

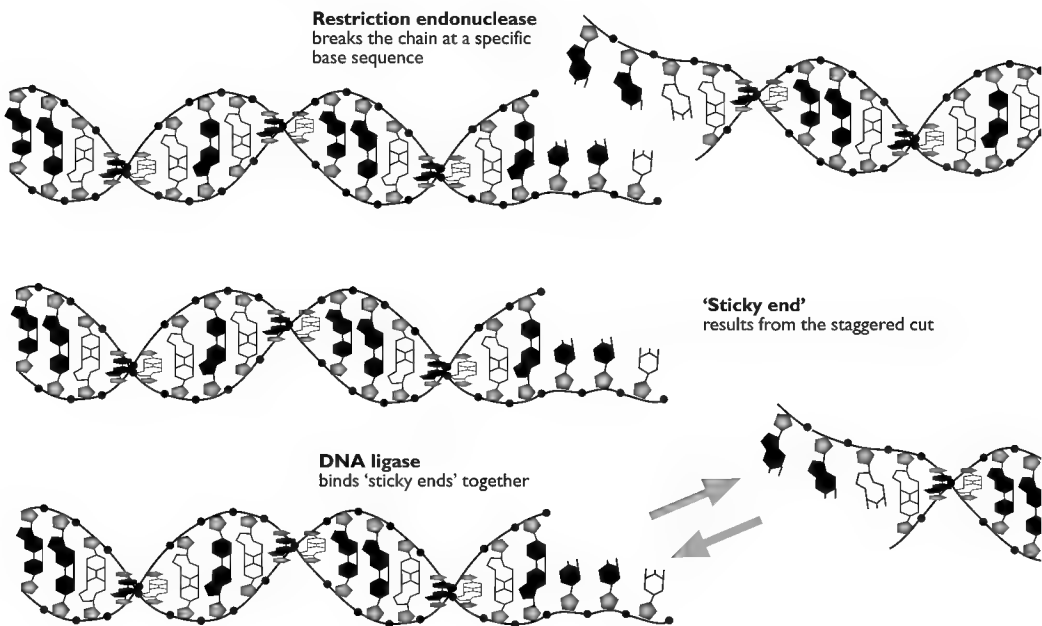
scissors to chop up DNA at specific base code sites into smaller polynucleotide fragments. Restriction enzymes are extracted from bacteria. In bacteria they serve to protect the organism from invading viral DNA. In gene technology they are used to cut DNA into fragments which can be separated and isolated. Often the cut leaves '**sticky ends**' which are exploited in another stage of the process.

DNA ligase

To 'ligate' is to join. DNA ligase is a joining enzyme. It is used to stick the sticky ends of DNA fragments together creating new combinations (**recombinant DNA**).

The techniques for transferring genes from one organism to another can be considered in five main stages.

Action of restriction and ligase enzymes



Isolation of the required gene

It is surprisingly easy to extract DNA from living cells as you may already have discovered in the laboratory. Using restriction enzymes and gel electrophoresis it is possible to separate and isolate different DNA fragments, even pure genes. Alternatively, genes can be extracted from cells in the form of mRNA as described above and converted into DNA using reverse transcriptase.

The use of a vector

A vector is a carrier. The most common vectors in gene technology are bacterial plasmids and

bacteriophage viruses, both of which can carry the required gene to the host cell.

Plasmids are rings of DNA found in bacterial cells which are not part of the main bacterial chromosome. They are easily transferred from one bacterial cell to another. Plasmids can be cut at specific sites using restriction enzymes to leave sticky ends. If the same enzyme is used on the isolated gene then the sticky ends will match and the two can be joined using DNA ligase, so that the plasmid contains the recombinant DNA.

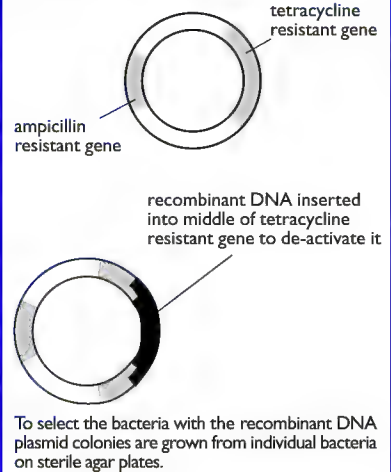
Bacteriophages are viruses which infect bacteria. They have a simple ring of DNA which they inject into the bacterial cell. This ring of DNA resembles a plasmid and can be modified in the same way to contain the required gene.

Transformation of the host cells

The vector is now brought into contact with the host cells, in the hope that some of the recombinant DNA might be taken up by the host cells. In order to discover whether or not the host cells have been transformed, **marker genes** (selection genes) are used. These are genes which are engineered into the vector alongside the required gene. They do not harm the host cell in any way but code for products which show up in an easily identifiable form in the host cell, confirming that the process of transformation is complete. One of the more common marker genes codes for antibiotic resistance. The transformed host cells can be isolated by introducing an antibiotic to the culture. All non-transformed cells are killed by the antibiotic, but the transformed cells survive as a result of the antibiotic resistant marker gene.

There has been some concern expressed about the use of antibiotic resistant genes. Opponents of this technique point to the risk of such genes escaping and entering disease organisms. Imagine the potential of a disease-causing bacterium, genetically modified to resist antibiotic treatment.

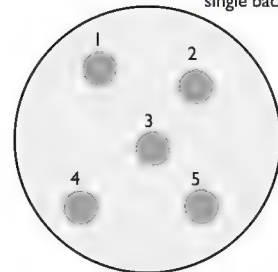
Inserting recombinant DNA



Replica plating

Plate A

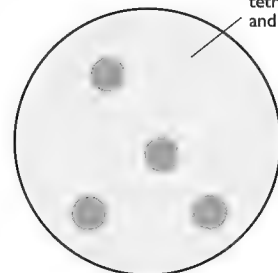
Five colonies, each grown from single bacteria



A sterile plate is pressed upon plate A and then pressed upon the sterile agar of plate B. This leaves plate A intact and passes the identical pattern of bacterial growth onto plate B.

Plate B

agar with tetracycline and ampicillin



Colony 2 has failed to develop, these bacteria must have the de-activated tetracycline resistant plasmid. These bacteria can be cloned and cultured from the colony growing on plate A.

Multiplication of transformed cells

Once a cell or group of cells has taken up recombinant DNA, the next step is to culture the cells in a suitable growth medium, allowing them to grow and multiply. In the case of transformed bacterial and yeast cells, this can be done on a large scale in industrial fermenters, allowing the product(s) of the new gene to be harvested and purified, as in the case of insulin, cofactor VIII and chymosin.

QUESTIONS RAISED by GENE TECHNOLOGY

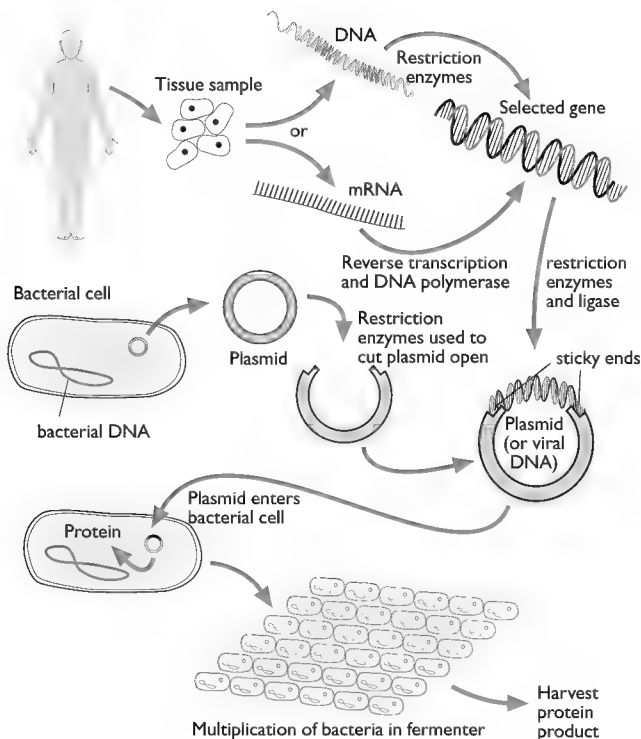
Gene technology raises many hopes for the future. It should be possible for example to expand the tolerance range and extend the habitat of many crop plants by engineering resistance to salinity, heat, cold, and wind. New varieties will compete better with weeds and be more tolerant of attack from insects and fungal disease. Nitrogen fixation will be enhanced, and renewable energy crops will begin to make a realistic difference to the greenhouse gas levels. Malnutrition may be overcome by staple food crops with a complete complement of essential amino acids, and new drugs will be developed from plant substances.

It also raises a number of questions about the way we want to live. Genetic modification has brought in the age of the designer plant, potatoes exclusively designed for oven chips, square tomatoes, straight bananas, salad vegetables, and fruits of every colour and texture. If there is a market for it, then the plant scientists will be able to engineer it. However, the accidental transfer of new genes into wild organisms could lead to herbicide resistant weeds and antibiotic resistant bacteria. Transgenic plants can also lead to transgenic pests and microbes.

The world population, currently about 5.5 billion, is expected to double to 11 billion by the year 2050. Given that 1 billion are already classified by international agencies as 'poor' and 500 million live in 'absolute poverty', it is clear that food production must be more than doubled in the next 50 years, and this must be achieved on a reducing area of fertile land.

Plant biotechnology, already moving at a rapid pace, offers some hope for the future. Plant biotechnology however, is largely the property of 'First World' independent companies, and 97% of the world's population increase will occur in the developing nations. Furthermore, environmental safety procedures, which evolve with the industry, exist in, and apply only to the countries which have the technology, yet transgenic products now have world wide distribution. A great deal of international cooperation, not to mention finance, will be needed to overcome the problem of safely transferring the products of the new technology to the countries which need them most. The human genome project which is analysing the sequence of bases in human DNA, also raises many problems to be dealt with. Certainly some inherited conditions and tendencies will be better understood, and various treatments developed. On the other hand the potential for abuse is enormous, including refusal of insurance cover to certain groups, employment vetting, immigration control and many others.

Genetic engineering



◆ CHECKPOINT SUMMARY

- ◆ Isolation of the gene to be manipulated is the first step
- ◆ It is easier to find and isolate the appropriate mRNA which has been transcribed (copied) from the relevant DNA, than it is to try and isolate the particular length of DNA itself.
- ◆ The enzyme reverse transcriptase can then be used to make a copy of the DNA from the mRNA.
- ◆ Plasmids (circular DNA) are isolated from bacteria.
- ◆ Plasmids are cut open by restriction enzymes and the length of DNA is inserted and the plasmid resealed by ligase enzymes.
- ◆ The genetically modified plasmids are then reintroduced into the bacteria.
- ◆ The bacteria multiply and produce the protein coded for by the inserted DNA.
- ◆ To identify those bacteria which have successfully taken up the modified plasmids marker genes are incorporated along with the required gene.
- ◆ Marker genes include those for antibiotic resistance so that the culture can be flooded with antibiotic and only the bacteria with the marker gene will survive.
- ◆ Two early successes with these techniques were the synthesis of human insulin for the treatment of diabetics, and blood clotting factor VIII for the treatment of haemophiliacs.

3.7

Non-communicable disease

Contents

The biological basis of heart disease

- ▼ Atheroma as the presence of fatty materials within the walls of arteries
- ▼ Explanation of the link between atheroma and the increased risk of aneurysm and thrombosis
- ▼ Myocardial infarction and its cause in terms of an interruption to the blood flow to the heart muscle
- ▼ Risk factors associated with coronary heart disease
- ▼ Blood cholesterol, cigarette smoking and increased blood pressure.

The biological basis of cancer

- ▼ The main characteristics of tumours and tumour cells
- ▼ The distinction between benign and malignant tumours
- ▼ Consideration should be given to the following aspects:
 - genes and the part they play in the control of normal cell growth
 - chemical carcinogens and radiation may damage DNA and cause mutations in the genes controlling growth
 - tumour cells failure to respond to normal growth regulating processes; metastasis and the invasion of other organs
 - the role of tumour suppressor genes in preventing tumour growth
- ▼ The incidence of lung and skin cancers in the United Kingdom. Factors which increase the incidence of these cancers. Interpretation of data showing the pattern of incidence and links with possible causal factors
- ▼ The moral and ethical issues associated with cigarette smoking and disease.

Introduction

The term 'non-communicable' or non-infectious disease refers to a broad category of diseases which includes all those conditions not caused by pathogens. It includes genetic diseases, mental diseases, self-inflicted diseases and the multifactorial degenerative diseases which include cancers and cardiovascular diseases.

Diseases in this latter sub-group have rapidly become the largest and most significant cause of illness and death across the Developed World during the twentieth century. Deaths from such diseases are also increasing rapidly in many developing countries, and in some have overtaken infectious diseases as leading causes of death. Whilst degenerative diseases mainly affect people in middle age or above, many of the diseases including cardiovascular disease, some cancers, diabetes, and obesity are the result of a life time of exposure to a range of risk factors.

The BIOLOGICAL BASIS of HEART DISEASE

Cardiovascular Disease

The cardiovascular system includes the heart and the blood vessels through which blood is pumped around the body. Cardiovascular disease is thus a term which covers several different diseases within this system, including **high blood pressure** (hypertension), **atherosclerosis** (narrowing of the arteries with fatty deposits) and **coronary heart disease** (atherosclerosis specifically affecting the coronary arteries which supply the heart). This latter disease may lead to fatal heart attack or **myocardial infarction**. This is the cause of death in 110,000 people in England and Wales each year.

Atherosclerosis

Atherosclerosis is a condition in which the large arteries of the body (especially the aorta, carotid arteries and coronary arteries) become hardened and narrowed by the build up of deposits inside the artery wall. These deposits, which are known as atheromata (plural of atheroma) are composed of cholesterol and other fatty materials, which come from the blood plasma. Whilst atherosclerosis is usually only found in adults, atheroma deposits may start to build up from childhood.

Although not fully understood, atheroma build up is thought to begin in areas of the arteries which have been damaged. Damage to the inner lining or endothelium, perhaps caused by high blood pressure (overstretching of the artery walls), or by the effect of chemicals derived from cigarette smoking, allows lipids (especially cholesterol) in the plasma to enter the artery wall. The lipids accumulate beneath the smooth muscle layer, appearing at first as small fatty streaks which later become fibrous and hardened as connective tissue builds up around the site. The region becomes known as a plaque. The artery wall will thus start to bulge into the lumen. The plaque may also damage the elastic and muscle tissues in the artery wall.

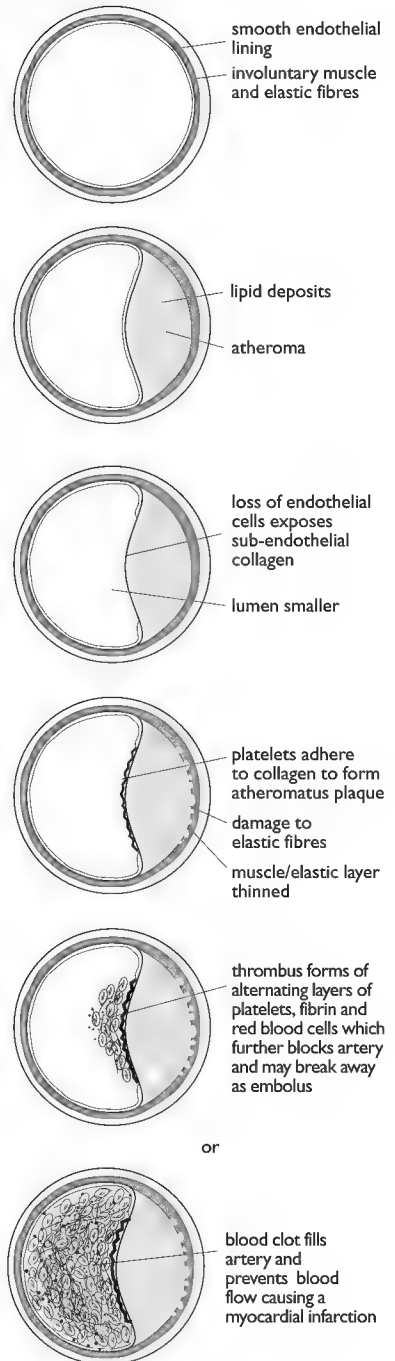
The effects of atherosclerosis: high blood pressure, aneurysm and thrombosis.

Atherosclerosis is a serious risk factor in many other cardiovascular conditions. Firstly it may lead to high blood pressure which is linked to the loss of elasticity in the arteries. Resistance to blood flow is increased, forcing the heart to pump blood at a higher pressure to reach all the tissues.

As discussed above, atherosclerosis may weaken the artery wall since the fibrous plaque disrupts the normal structure of the wall. In particular the layers of muscle and elastic tissue (the tunica media) may become thinner. In some cases this can lead to an expansion of the artery known as an aneurysm (a balloon like swelling of the vessel). This may burst, and since the artery most affected by aneurysms is the aorta, this is usually fatal if immediate surgical help is not available. High blood pressure may be a contributing factor in the development of aneurysms.

A further complication of atherosclerosis is that of thromboses or internal blood clots. In diseased arteries, the roughened endothelium covering a plaque may rupture, allowing blood to flow into the

Thrombosis Development in Coronary artery



plaque. Platelets from the blood are attracted to the damaged area and stick to the artery wall where they release clotting chemicals. Gradually a thrombus will form. Similar mechanisms to those involved in blood clotting at a wound site are involved (see section 12.3) Once formed the thrombus may cause damage in one of two ways. It may completely block the artery preventing blood flow to areas of tissue. If this occurs in a coronary artery this can lead to myocardial infarction, which occurs when parts of the heart muscle are starved of oxygen and glucose. Without oxygen and glucose the muscle tissue cannot respire and thus has no ATP for contraction of the muscle fibres. The heart then fails to contract properly and death may follow.

Myocardial infarction may occur suddenly without prior warning or may be preceded by pain in the chest region (angina), this pain is caused by narrowing of the coronary arteries and their inability to supply adequate oxygen to heart muscle especially during exercise.

In other cases the clot may detach itself from the artery wall and enter the circulation. Known as an **embolus** this clot may then block a vessel in another part of the body. Often they lodge in the pulmonary artery, or in vessels of the brain leading to a stroke. Such blockages of vessels can lead to death, or tissue damage, but in other cases may be treated by giving drugs to break down the clot, e.g. warfarin or heparin which reduce blood clotting.

Risk Factors in coronary heart disease

In common with other non-communicable diseases, coronary heart disease (CHD) is not caused by one factor but has a number of 'risk factors' associated with it. Each risk factor increases the risk of developing coronary heart disease, although none of them are sufficient to bring about the disease on their own. In some cases the link between the factor and the risk of CHD is not well understood. Risk of CHD may build up by long exposure to known risk factors many of which are avoidable.

The following have been identified as major risk factors:

High blood cholesterol level. There is a strong positive association between high levels of cholesterol and other saturated fats in the blood and risk of CHD. Such lipids do not exist free in the blood but in compounds of lipid and protein known as Low Density Lipoproteins (LDL). Such high levels of LDLs have dietary causes (diets high in saturated fats such as are found in butter and some meats), and genetic causes, by which some people will naturally have high levels of LDL even if their diet is low in cholesterol.

Interestingly the consumption of some polyunsaturated fats which travel in the blood as high density lipoproteins seem to have a protective effect on CHD. It is thought that these HDLs reduce plasma levels of LDLs perhaps through altering the metabolism of cholesterol in the body. Polyunsaturated fats are found in many vegetable oils, including olive oil. The relative proportions of HDLs and LDLs are important.

Cigarette smoking. Smoking is thought to be responsible for up to 40% of all deaths from CHD. A person who smokes 25 cigarettes per day is up to 15 times more likely to develop CHD than a non-smoker. The reasons for this are still not well understood, but the following effects have been noted:

Carbon monoxide (CO) found in tobacco smoke combines irreversibly with haemoglobin in red blood cells producing carboxyhaemoglobin. This compound cannot carry oxygen so oxygen delivery to tissues may be reduced. This may in turn cause heart rate and blood pressure to increase. Also, carbon monoxide, along with nicotine, is toxic to the endothelial lining of arteries and is thought to be one factor which may cause damage to this lining and allow cholesterol to enter the vessel wall causing development of atheroma.

Nicotine, the addictive component of cigarette smoke has a number of effects on the cardiovascular system. It causes the blood vessels to constrict, increasing blood pressure and heart rate. This puts extra strain on the heart and can be serious for the person who has already had a coronary attack. Nicotine also increases the concentration of fatty acids in the blood, which may contribute to the deposition of atheroma. This along with the fact that nicotine makes blood platelets more sticky, increases the possibility of blood clotting inside blood vessels leading to thrombosis. At the same time more fibrinogen (one of the proteins involved in blood clotting) is produced, while lower levels of enzymes which break down blood clots are found. Blood clots formed in the coronary arteries may block the artery leading to a myocardial infarction or heart attack. Nicotine also makes the heart more irritable and can precipitate dangerous irregularities in heart beat.

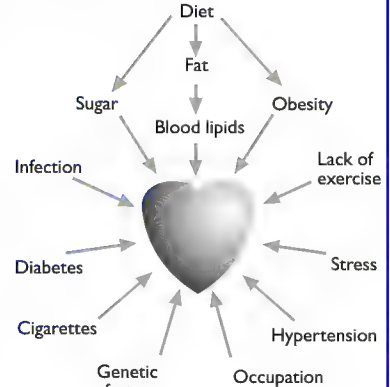
High blood pressure is closely linked to many other risk factors in CHD including smoking, obesity, diabetes, stress, high salt intake and lack of exercise. It is thus difficult to consider the effects of high blood pressure in isolation.

Lack of exercise is often considered to be one of the most important and most easily corrected risk factors in CHD. Exercise in itself can increase the strength of the heart muscle and decrease the resistance of blood flow in vessels. It also helps to prevent obesity, reduce stress and is often associated with diets lower in saturated fats.

Age and sex. Whilst CHD can affect young people it is relatively rare in those younger than about 40 years. It must be stressed, however, that accumulation of atheroma may begin in childhood, but its effects in terms of CHD may not be seen for several decades. CHD is much more common in men than women in middle age. This effect decreases in post-menopausal women and a protective effect of oestrogen has been suggested.

It should be noted that with the exception of age, sex and genetic predisposition, all of the major risk factors for CHD can be avoided.

Heart disease factors



■ CHECKPOINT SUMMARY

- ◆ Atheroma (pleural atheromata) are fatty deposits of cholesterol and other fatty materials in the walls of large arteries (especially the aorta, carotid arteries and coronary arteries) leading to the condition atherosclerosis.
- ◆ Lipids accumulate beneath the smooth muscle layer, appearing at first as small fatty streaks which later become fibrous and hardened as connective tissue builds up around the site. The region becomes known as a plaque.
- ◆ Atherosclerosis may lead to high blood pressure which is linked to the loss of elasticity in the arteries, and can lead to an expansion of the artery known as an aneurysm, and thromboses or blood clots which may occur in any diseased artery.
- ◆ Once formed the thrombus may completely block a coronary artery this can lead to myocardial infarction, which occurs when parts of the heart muscle are starved of oxygen and glucose.
- ◆ In other cases the clot may detach itself from the artery wall and enter the circulation. Known as an embolus this clot may then block a vessel in another part of the body. Often they lodge in the pulmonary artery, or in vessels of the brain leading to a stroke.
- ◆ Risk factors associated with coronary heart disease include, high blood cholesterol level, cigarette smoking and its associated carbon monoxide (CO) and nicotine, high blood pressure, lack of exercise, and is much more common in middle aged men.

The BIOLOGICAL BASIS of CANCER

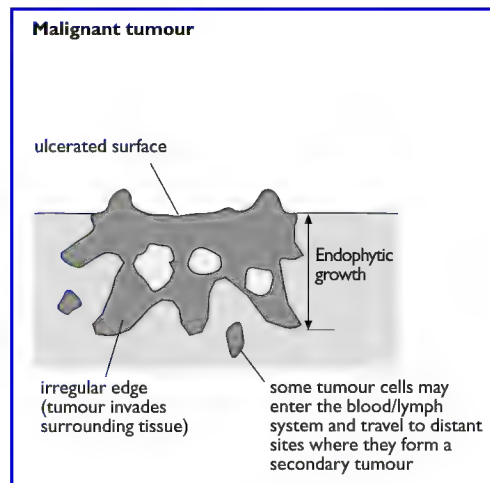
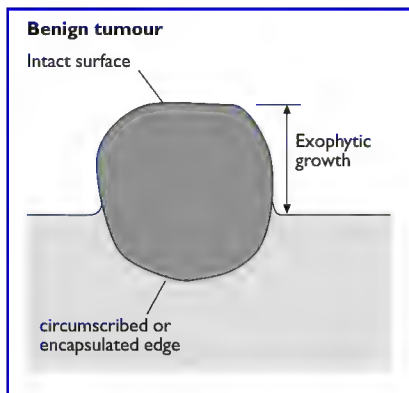
Introduction

In 1999 7.3 million people worldwide died from cancers, of whom 1 million were in high income European countries. In such countries cancers now rank as the second most important cause of death (behind cardiovascular disease), with by far the most common being lung cancer. Other cancers which cause large numbers of deaths are prostate cancer in men, breast cancer in women and cancer of the colon in both sexes. Cancer is often classified as a degenerative disease, so it is not surprising that the vast majority of these deaths occur in older adults. It should be noted that cancer is not a single disease but rather a complex group of several hundred different diseases which share common characteristics. Chief amongst these is the presence of unregulated cell growth leading to the development of tumours.

The main characteristics of tumours and tumour cells.

A tumour is an abnormal mass of tissue, the growth of which is uncoordinated and exceeds that of normal tissue. Tumours are divided into **benign** and **malignant**. The principal difference is that malignant tumours invade their surrounding tissues and spread to distant sites via the blood stream to form secondary tumours, known as **metastases**; while benign tumours usually grow at a slower rate, they never invade organs or blood vessels/lymphatics nor do they metastasize. Such tumours are more easily removed during surgery than malignant ones.

Tumour cells, whether benign or malignant, differ from normal cells both in behaviour and appearance.



Differences in behaviour of tumour cells

Tumour cells often grow rapidly and out of control. When normal cells are grown in culture, for example on an agar plate, they will divide and spread out over the surface of the plate. Once they have covered the plate in a layer one cell thick, cell division stops. In contrast, malignant cells grown under the same conditions continue to grow in a heaped up multilayered form.

Differences in appearance of tumour cells

Although tumour cells may arise in many different sites in the body, they have the following differences to normal cells:

- ▼ wide variation in the shape and size of the cell
- ▼ large nucleus when compared to the size of the cell, which stains darkly.

As a general rule benign tumour cells resemble the normal cell type from which they have arisen, while malignant tumour cells do not.

Classification of tumour cells

Tumours are classified firstly as benign or malignant, and secondly by their organ of origin, such as the breast, or even by their original cell type.

Identification is usually made on the basis of a biopsy. This is a representative fragment of the tumour removed and examined by a pathologist under the microscope. Once the type of tumour has been identified, the likely course of the disease (prognosis) and treatment can be determined.

Causes of cancer

Chemical carcinogens and radiation may damage DNA and cause mutations in the genes controlling growth.

Chemical carcinogens

A chemical that increases the risk of developing a cancer is referred to as a **carcinogen**, or as being carcinogenic. These include chemicals found in the tar of cigarette smoke, in car exhaust fumes, and in low levels in some common foods and food additives.

Radiation

Ultra-violet light, gamma rays and X-rays are also carcinogenic. Radiation damages DNA and malignant transformation is more likely.

The Role of Genes in Normal Cell Growth and Cancer

The normal growth of cells is regulated by growth factors. These are generally polypeptides present in the blood plasma, that bind to receptors on the cell surface. This **growth factor-receptor complex** then activates an enzyme which in turn will activate second messengers. These second messengers stimulate factors that start DNA transcription, and the synthesis of proteins necessary for cell growth and division.

Cancer is a term used to describe a huge range of diseases. However, at the molecular level they all share common features. The uncontrolled growth which exceeds that of normal tissue, results from mutations in the genes that encode for proteins regulating the normal growth of cells and the cell cycle.

The majority of mutations in the genome have no influence on cell growth. Indeed some mutations result directly in the death of the cell. However, some gene mutations result in the cell escaping from the normal controls that regulate their growth and division. Significant mutations are rare events and often several mutations in a single gene are required before a tumour develops. However, one single transformed cell can give rise to a tumour through repeated cell division. A tumour is therefore usually monoclonal: all the cells are derived from a single mutated precursor cell.

Tumour suppressor genes encode for proteins which suppress cell growth in the normal cell. It is thought that many of these genes encode proteins which suppress DNA synthesis. Thus a mutation in one of these suppressor genes may result in production of a defective protein and loss of suppression of cell growth and division, resulting in the development of a tumour.

Programmed cell death (apoptosis) is part of the normal cell cycle and involves many proteins including enzymes. Disruption of the genes encoding these proteins may cause cells to escape programmed cell death and continue to divide to form a tumour.

Lung Cancer: incidence and causes.

Lung cancer accounts for 19% of all cancers and 27% of cancer deaths in Britain, and causes an estimated 1.2 million deaths worldwide in 1999. It is the most common cancer in British men, although the incidence is no longer rising. Whilst breast cancer remains the most common cancer in women, the incidence of lung cancer in this group is rising. The reasons for this are discussed below. Overall, lung cancer causes over 40 000 deaths per year in the United Kingdom.

There is indisputable evidence that most lung cancer is caused by smoking; there is also an association with radiation exposure, asbestos and air pollution; and the evidence that passive smoking causes cancer is growing.

The link between smoking and lung cancer has been established by the work of Sir Richard Doll. He demonstrated the strong links between smoking and lung cancer, chronic bronchitis, and emphysema in a study which began in the 1950s. He used a technique known as the **prospective study** in which he identified his study group first, and then recorded the deaths from smoking-related diseases which occurred in the group over a number of years.

Smoking among secondary school children 1996

Increases shown in bold.

Boys	1982	1996
Age 11	1%	1%
Age 12	2%	2%
Age 13	8%	8%
Age 14	18%	13%
Age 15	24%	28%
Total	11%	11%

Girls	1982	1996
Age 11	0%	0%
Age 12	1%	4%
Age 13	6%	11%
Age 14	14%	24%
Age 15	25%	33%
Total	11%	15%

His study population were male general practitioners (GPs) who were happy to be involved in a medical trial, were of broadly similar socio-economic groups, had similar lifestyles, occupation, and exposure to other risk factors. He recorded the number of cigarettes smoked per day in this group and over a period of many years recorded deaths from lung cancer, bronchitis and emphysema.

He found that heavy smokers (more than 25 cigarettes per day) had a risk of lung cancer 25 times higher than non-smokers, and 10 cigarettes per day increased the risk 10-fold over that of non-smokers. Pipe smokers were at a lower risk, presumably because of lower amounts of inhalation. Risk of lung cancer in ex-smokers declined, so that 13 years after quitting smoking, the risk was the same as that of a non-smoker. Other factors (such as exposure to pollutants) were considered before the data was analysed.

Atypical cells may be seen in smoker's lungs, and in lung tissue close to tumours. These are probably a reflection of damage to DNA caused by smoking resulting in mutations. If the person continues to smoke, further genetic changes will occur and they will be at high risk of developing cancer.

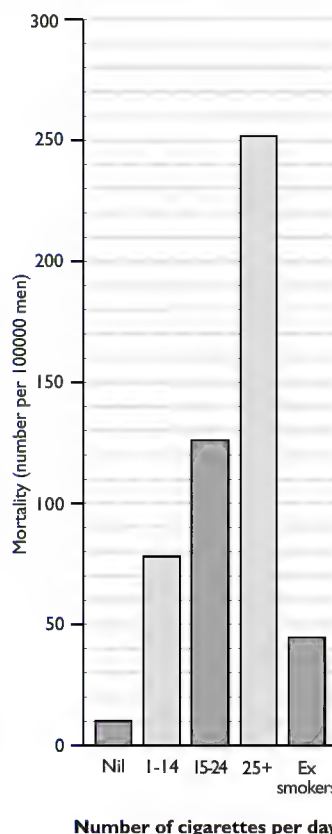
Skin cancer: incidence and causes

There are three main types of skin cancer, defined by the cell type from which they arise. Two types: **basal cell carcinoma** and **squamous cell carcinoma** are both very common. The main factor increasing their incidence is exposure to sunlight. It is thought that ultraviolet light in the sun's rays causes direct damage to DNA.

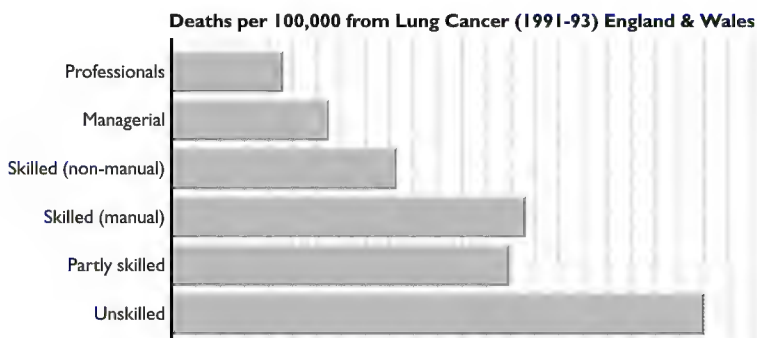
Several features of these two cancers implicate sunlight as an important cause.

- ▼ They most commonly occur in elderly patients, with a lifetime of exposure to the sun (and thus the chance to accumulate mutations in their DNA).
- ▼ The tumours are most commonly found on sun exposed areas of the body, e.g. the face and arms.
- ▼ They are more common in light skinned people, who have less melanin, allowing more ultraviolet light to reach and damage skin cells.

Graph to show death rates from lung cancer according to smoking habits



Deaths from lung cancer according to socio-economic group



- ▼ These cancers are more common in individuals who work outside, than in those who have office jobs.
- ▼ These cancers are more common in patients who undergo ultraviolet light therapy for treatment of other conditions, such as psoriasis.

The third type of cancer is malignant melanoma. Although rare, with 6 new cases per 100 000 per year in the UK, it is still the most common cancer in the 20-39 age group. Large epidemiological studies similar to those outlined for smoking and lung cancer, have established that sunlight exposure is strongly associated with malignant melanoma. Fair skinned individuals living close to the equator are most at risk. Thus while the incidence of melanoma in Black populations is only around 0.6 per 100 000 per year, it is as high as 33 per 100 000 per year in those of European descent living in Queensland, Australia.

The incidence of melanoma in the UK is rising at an alarming rate, and this almost certainly reflects changing attitudes towards sun exposure. Travel abroad is becoming more affordable, and many people strive hard for a tan, in the mistaken belief that it denotes healthiness. In contrast to the two other types of skin cancer outlined above where total sun exposure appears to be the main risk factor, in the case of melanoma, it appears that the risk is increased by episodes of severe burning.

◆ CHECKPOINT SUMMARY

- ◆ Cancer is not a single disease but rather a complex group of several hundred different diseases which share common characteristics. Chief amongst these is the presence of unregulated cell growth leading to the development of tumours.
- ◆ A tumour is an abnormal mass of tissue, the growth of which is uncoordinated and exceeds that of normal tissue.
- ◆ Tumours are divided into benign and malignant.
- ◆ Malignant tumours invade their surrounding tissues and spread to distant sites via the blood stream, the lymphatics or across body cavities to form secondary tumours, known as metastases
- ◆ Benign tumours usually grow at a slower rate, they never invade organs or blood vessels/lymphatics nor do they metastasize.
- ◆ Tumour cells have the following differences to normal cells: wide variation in the shape and size of the cell large nucleus when compared to the size of the cell, which stains darkly.
- ◆ As a general rule benign tumour cells resemble the normal cell type from which they have arisen, while malignant tumour cells do not.
- ◆ Causes of cancer include chemical carcinogens and radiation which may damage DNA and cause mutations in the genes controlling growth.
- ◆ Carcinogens are chemicals that increases the risk of developing a cancer; and include chemicals found in the tar of cigarette smoke, in car exhaust fumes, and in low levels in some common foods and food additives.
- ◆ Ultra violet light, gamma rays and X-rays are also carcinogenic. It damages DNA and malignant transformation is more likely.
- ◆ The uncontrolled growth seen in cancer, results from mutations in the genes that encode for proteins regulating the normal growth of cells and the cell cycle.
- ◆ A mutation in suppressor genes which code for proteins which suppress cell growth in the normal cell may result in a tumour.
- ◆ Mutations in genes which encode for proteins which control programmed cell death may cause cells to continue to divide to form a tumour.
- ◆ Lung cancer accounts for 19% of all cancers and 27% of cancer deaths in Britain and causes an estimated 1.2 million deaths worldwide in 1999.
- ◆ Skin cancer is correlated with radiation damage by UV light in sunlight, the incidence of melanoma in Black populations is only around 0.6 per 100 000 per year; but as high as 33 per 100 000 per year in those of European descent living in Queensland, Australia.

The moral and ethical issues associated with cigarette smoking and disease

This is a highly complex and controversial topic. The issues can most simply be divided into three areas: the impact of smoking on society, the individual smoker and the individual non-smoker.

Smoking is an contributing factor in around 50% of all hospital admissions. Apart from lung cancer, there is strong evidence linking smoking to bladder, laryngeal, mouth, oesophageal, stomach cancers and many other tumours. Bronchitis, emphysema, heart disease and peripheral vascular disease are much more common in smokers than non-smokers. Many people feel that smoking is a self inflicted disease, and therefore not a burden that the NHS and society should carry the cost of.

Others will argue that smokers have the same rights to the NHS as non-smokers. Many will argue that they started smoking before the risks of smoking were known, and they are now addicted and unable to give up. They would also claim that obesity and alcoholism are similarly self inflicted but few suggest that these groups should suffer discrimination. There is also truth in the claim that the heavy taxation on cigarettes contributes to the National Health Service (NHS). A lifetime of smoking (and increased taxation) would more than cover the extra burden of this group on the NHS. Also, the decreased life expectancy of smokers means that fewer live to retirement age when they can claim a pension from the State.

On an individual level, smokers argue that smoking is a legal pastime, and every individual has a right to smoke. However, there is emerging evidence that breathing in other people's smoke (passive smoking) increases a non-smokers risk of smoking related diseases. Non-smokers have used this to push through legislation restricting smoking in many public places.

Perhaps it is more generally agreed by society that individuals should be made fully aware of the risks of smoking, and those who wish to give up should be helped to do so. Despite health education programmes, and the warnings which now appear on all cigarette packets and advertising material, smoking remains widespread. The British Government has set targets for a 30% reduction in smoking related diseases in men and a 15% reduction in women by 2010. It is essential that such programmes target young smokers since the earlier a person starts smoking, the greater is their risk of death from related illnesses.

3.8

Disease may be diagnosed by a variety of techniques

Contents

DNA probes and diagnosis

- ▼ The use of DNA probes to identify the presence of specific genes associated with human disease
- ▼ This process in such detail as to show that:
 - restriction enzymes are used to cut DNA into fragments
 - electrophoresis is used to sort fragments according to charge and size
 - radioactive DNA probes are used to locate specific DNA fragments.

Enzymes and diagnosis

- ▼ Disease and changes in the concentration and distribution of enzymes in the body
- ▼ Pancreatitis and the increased concentrations of digestive enzymes in the blood or a decrease in the concentration of these enzymes in the gut
- ▼ The high sensitivity and specificity of enzymes allowing their use as analytical reagents. The use of glucose oxidase and peroxidase in testing for glucose.

Introduction

As discussed previously, diseases have a wide range of causes. Some, such as malaria and schistosomiasis are caused by pathogens; others, such as cystic fibrosis and PKU are caused by faulty genes; yet others, including CHD, cancers and diabetes result from a range of interacting environmental and genetic factors. Whatever the cause of the disease, correct diagnosis is essential if the disease is to be treated effectively.

The USE of DNA PROBES to IDENTIFY the presence of specific genes associated with human disease

Genetic screening is the name given to a range of procedures which aim to detect the presence or absence of a particular gene or chromosome mutation in an individual. It is most commonly used in the detection of genetic diseases such as sickle cell anaemia, cystic fibrosis, haemophilia, thalassaemia, and Duchenne muscular dystrophy, each of which are caused by a single gene mutation at one locus. In combination with techniques for amplification of DNA (PCR) modern screening techniques can use DNA from just a single cell to screen for several different genetic diseases. This may be done using DNA from adults (carrier detection), from a developing foetus in utero (prenatal screening), or from a fertilised ovum prior to implantation in IVF (pre-implantation screening).

Genetic screening involves a number of stages:

- ▼ Extraction of the DNA from cells (e.g. white blood cells, cheek cells, gametes, the embryonic tissues of the placenta, or undifferentiated cells in the embryo)
- ▼ Amplification of the DNA using polymerase chain reaction (PCR). This allows many copies of the required section of DNA to be produced from a single DNA molecule.
- ▼ Use of specific restriction enzymes to cut the DNA at sites within and around known gene loci.
- ▼ Use of gel electrophoresis to split the DNA fragments up on the basis of their size.
- ▼ Southern blotting and use of radioactive DNA probe to locate the fragments of DNA.
- ▼ Autoradiography to create an image of the DNA pattern for interpretation

Restriction enzymes

Restriction enzymes or restriction endonucleases 'cut' DNA at specific base sequences. Such enzymes will 'recognise' particular DNA sequences known as recognition sites, attach to these, and 'cut' the DNA. The process of cutting the DNA is known as **cleavage**, and the fragments of differing lengths produced in this way as **restriction fragment length polymorphisms** (RFLPs).

The natural function of bacterial restriction enzymes is to cut out sections of viral DNA which have entered the bacterial DNA, but in genetic screening and other genetic techniques they are used to cut a length of DNA into smaller pieces. There are several thousand types of restriction enzymes now in use whose names reflect the bacterial species from which they were extracted: thus EcoRI is derived from *E.Coli* and HindI from *Haemophilis influenzae*. Each restriction enzyme is specific to one base sequence and will digest or 'cleave' the nucleic acid (DNA) whenever such a site is encountered but at no other point. Examples of some restriction enzymes and their sites are shown below. It should be noted that some cut straight through both strands of DNA creating 'blunt ends', whilst others produce a staggered cut or 'sticky end' which is more useful in genetic engineering.

The significance of restriction enzymes in genetic screening lies in the fact that many restriction sites are located within or close to genes of interest. This means that mutations of these genes, which alter the DNA sequence, may destroy or add restriction sites. Under these circumstances DNA from individuals with the mutant gene will be cut into different length RFLPs than DNA from normal individuals when incubated with the same restriction enzyme. These length differences allow the detection of mutants using electrophoresis discussed below.

Enzyme	Source Organism	Restriction Site
EcoRI	<i>Escherichia coli</i>	↓ -G-A-A-T-T-C- -C-T-T-A-A-G- ↑
EcoRII	<i>E. coli</i>	↓ -G-C-C-T-G-G-C- -C-G-G-A-C-C-G- ↑
Hae III	<i>H. aegyptius</i>	↓ -G-G-C-C- -C-C-G-G- ↑
HpaII	<i>H. parainfluenzae</i>	↓ -C-C-G-G- -G-G-C-C- ↑
SmaI	<i>Serratia marcescens</i>	↓ -C-C-C-G-G-G- -G-G-G-C-C-C- ↑

Electrophoresis

This is a technique used in the analysis of DNA which has been cut into fragments of differing sizes by restriction enzymes. It is based on the principle that smaller fragments of DNA will migrate through a gel faster than larger ones when a direct electric current is applied across the gel (which is in a buffer solution). The reason for this is that the larger fragments have more difficulty moving through the dense gel. All fragments will however move as they are negatively charged and move to the positive terminal.

To perform electrophoresis a gel is made from agarose (a polysaccharide) and placed in a tank of buffer solution. DNA samples that have been treated with restriction enzymes are loaded into wells in the gel along with a dye solution. This ensures that all have equal electrical charge and that their position can be marked. A direct current is passed through the buffer solution causing the DNA to move through the gel, marked by the dye. Once the dye front has migrated about three quarters of the way down the gel the current is switched off. The position of DNA fragments on the gel cannot be seen at this stage: further steps are required for this. These are described below.

Southern Blotting

The first stage in allowing visualisation of the DNA fragments is known as Southern Blotting. There are two basic stages involved: firstly the DNA on the gel needs to be heated to cause the two strands of the helix to unwind making single stranded DNA (this is crucial as if the DNA is not single stranded it will not bind to the DNA probe);

secondly this single stranded DNA is transferred from the gel to a nitrocellulose filter or a nylon membrane. The membrane will bear an exact copy of the gel in terms of the positions of DNA fragments.

DNA probes

A DNA or gene probe is a single stranded DNA sequence usually 12 nucleotides or more in length which is complementary to a stretch of known DNA (e.g. part of a disease gene) on a chromosome. They are usually radioactively labelled using ^{32}P (the radioactive P is incorporated into the nucleotides which are used to make the probe). The probe is added to the nitrocellulose filter/nylon membrane (produced via Southern blotting) where it will attach to DNA of a complementary sequence. This is known as **hybridisation**. For example, if a particular gene contained the sequence: C C C T T C T T T C C C A A T A T then a gene probe would be made as follows.

DNA SEQUENCE: C C C T T C T T T C C C A A T A T

DNA PROBE G G G A A G A A A G G G T T A T A

The probe will bind to any region of DNA with this sequence as the nucleotides of the probe pair up with their complementary bases on the DNA. Once this has occurred excess probe is washed off the filter.

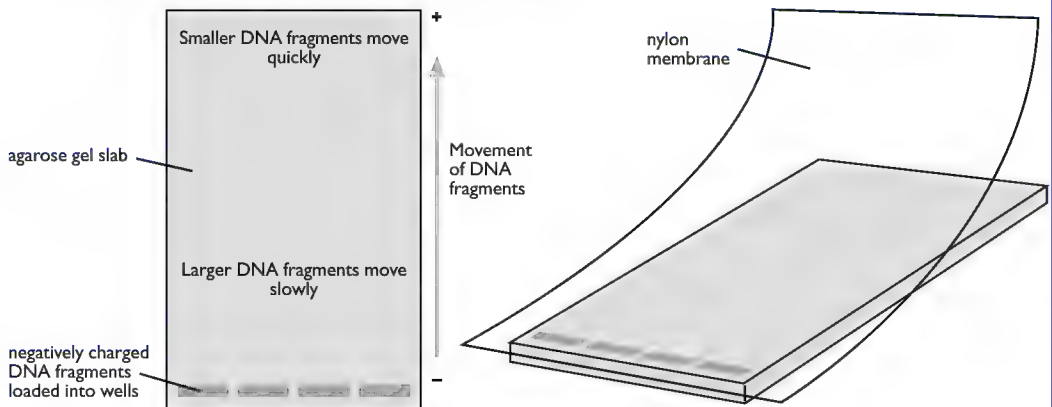
Autoradiography

In order to see the bound DNA probe and hence detect the position of DNA fragments separated in the original gel, an X-ray film is placed over the nitrocellulose filter. At sites where the gene probe has bound to DNA fragments the radioactive ^{32}P will create a black band on the film.

Analysis works as follows: a black band at the bottom end of the picture will correspond to small DNA fragments to which the probe has bound. Conversely, black bands at the top of the picture reveal large fragments to which the probe has bound. If a mutant gene is missing a restriction site which is present in a normal gene, then the mutant DNA will have travelled a shorter distance than the normal DNA. The positions of the bands can be compared to known 'control' DNA patterns or to the movement of DNA markers.

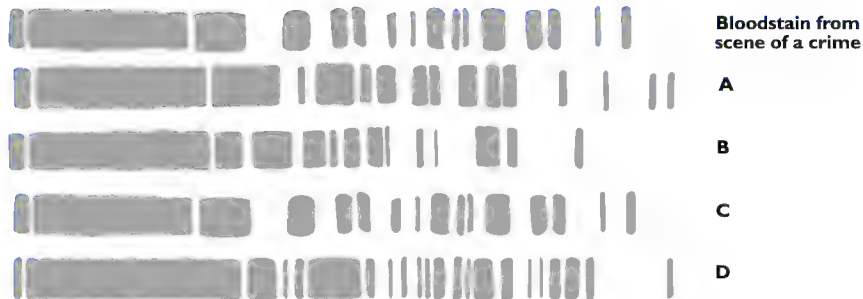
Stages in genetic fingerprinting

- 1 Extracted DNA e.g. from white blood cells, semen etc.
- 2 DNA cut into pieces by **Restriction Enzymes**
- 3 DNA pieces are separated by **Gel Electrophoresis** this takes 24 hours



- 4 **Southern Blotting**, the DNA fragments are transferred (blotted) onto a nylon membrane laid on top of the gel slab.
- 5 **Gene Probe Treatment** - the nylon membrane is put into a tank of single strand DNA's (probes) which are complementary to specific core DNA sequences and bind to them. The probes may be radioactive or fluorescent. Excess probes are washed off
- 6 X Ray film is placed on the membrane and the typical bands appear where the presence of **radioactive/fluorescent probes** fog the film.
- 7 The X Ray film is processed to form the **DNA Fingerprint** with its characteristic 'barcode' pattern of dark bands.

Genetic Fingerprinting - Autoradiograph bands



Was individual A,B,C or D more likely to have been present at the scene of the crime?



Which male is the child's father?

Enzymes and disease diagnosis

Diseases can result in changes in the concentration and distribution of enzymes in the body. Therefore the measurement of enzyme levels (enzyme assays) in body fluids can provide clinicians with valuable information about a range of diseases. This technique may be used to diagnose some of the inherited metabolic disorders where lack of a particular enzyme is the cause of the disease, e.g. very low levels of the enzyme glucose-6-phosphate dehydrogenase (G-6-P-D) in liver cells. Enzyme assays may also be used to detect changes in enzyme levels which reflect a disease process in the body, e.g. pancreatic disease (measurement of amylase, lipase and trypsin), hepatic (liver) disease (measurement of cholinesterase, alkaline phosphatase, and transaminases) and coronary heart disease (measurement of creatinine kinase). Levels of enzymes measured (usually in serum) are compared to known reference ranges for healthy individuals. In most cases such enzyme assays are used in conjunction with other investigations.

Pancreatitis

The pancreas secretes various digestive enzymes, proteases, lipases and amylases in an alkaline fluid. Some of these enzymes, notably the proteases are secreted in an inactive form and only activated in the duodenum.

Acute pancreatitis is a disease of the pancreas caused by premature activation of the protease enzymes which leads to digestion of the cells of the pancreas. The underlying causes of this are many and include gallstones, and exposure to excess alcohol. Symptoms of the disease are severe pain in the upper abdomen accompanied by nausea and vomiting. These symptoms are not very specific so doctors require results from specific tests of enzyme levels before pancreatitis can be diagnosed. Chronic (recurring) pancreatitis, usually caused by excess alcohol, can lead to permanent damage to the pancreas with implications for digestion and glucose regulation.

Acute pancreatitis can be diagnosed by measuring the levels of the pancreatic enzymes amylase and lipase in serum (blood plasma minus large proteins). Both enzyme levels are markedly increased since the cells which produce them are being destroyed allowing the enzymes to escape into the blood.

Chronic pancreatitis can be diagnosed by measuring the level of pancreatic enzymes in the intestine. Enzyme assays for the protease enzyme chymotrypsin may also be performed on faeces. In all cases the levels of pancreatic enzymes are markedly lower than in normal patients. One effect of the low lipase levels is to allow fats to pass through the intestine without being digested: this results in fat being present in the faeces.

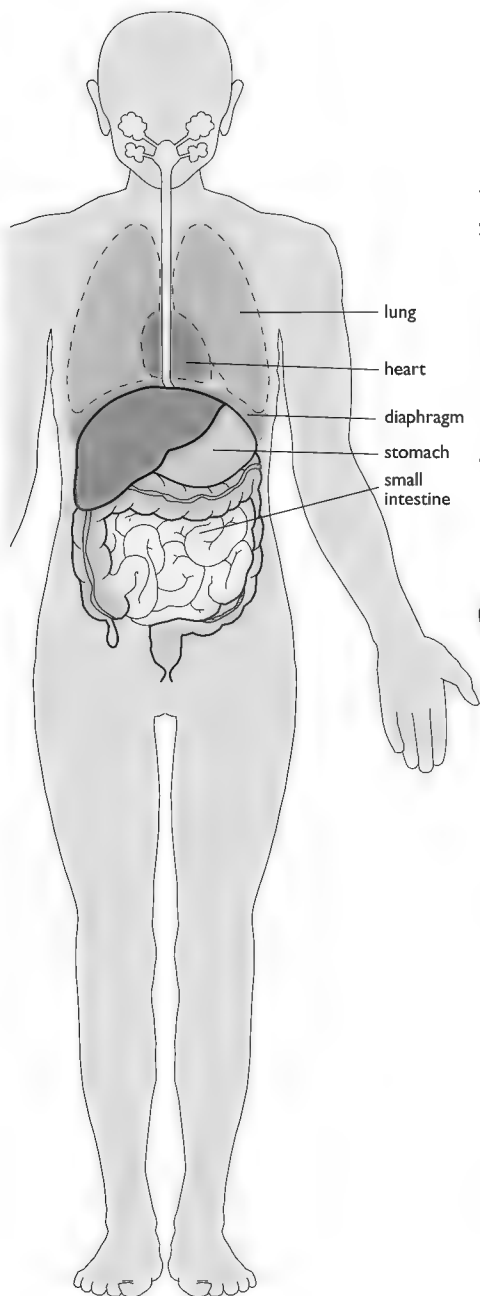
Repeat measurements of the pancreatic hormones in serum, intestine and faeces may be used to assess the course of pancreatitis and its response to treatment.

Raised levels of enzymes within the body may thus be an important diagnostic sign and a useful indicator of progression in some diseases. In other diseases the levels of biological molecules such as glucose or cholesterol are raised and can similarly be used in diagnosis or monitoring.

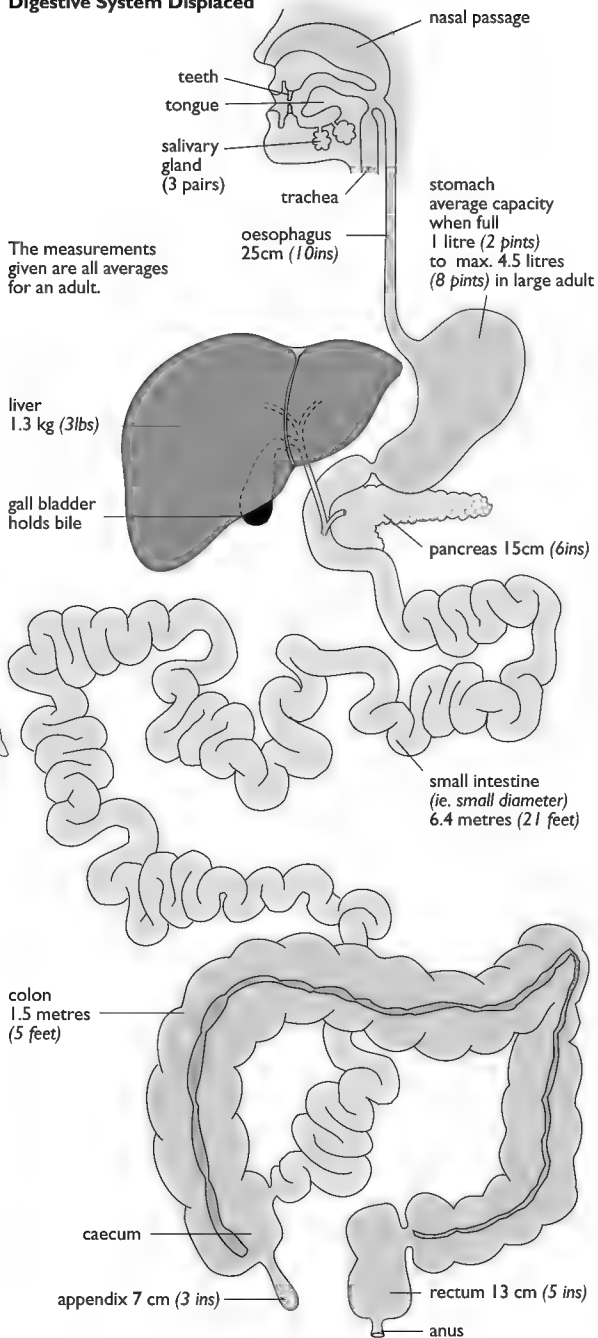
◆ CHECKPOINT SUMMARY

- ◆ A DNA probe (gene probe) is a single stranded DNA sequence usually 12 nucleotides or more in length which is complementary to a stretch of known DNA (e.g. part of a disease gene) on a chromosome.
- ◆ The use of DNA probes in diagnosis involves the following steps:
- ◆ Extraction of DNA from cells (e.g. white blood cells)
- ◆ The production of many copies of the required section of DNA (amplification) using the polymerase chain reaction (PCR)
- ◆ Use of specific restriction enzymes to cut the DNA at sites within and around known gene loci.
- ◆ Use of gel electrophoresis to split the DNA fragments up on the basis of their size.
- ◆ Southern blotting and use of radioactive DNA probe to locate the fragments of DNA.
- ◆ Autoradiography to create an image of the DNA pattern for interpretation
- ◆ Diseases can result in changes in concentration and distribution of enzymes in the body.
- ◆ The measurement of enzyme levels (enzyme assays) in body fluids can provide clinicians with valuable information about a range of diseases.
- ◆ Acute pancreatitis is a disease of the pancreas in which the protein digesting enzymes (proteases) produced by the pancreas become active within the pancreas and damage it.
- ◆ Acute pancreatitis can be diagnosed by measuring the levels of the pancreatic enzymes amylase and lipase in serum (blood plasma minus large proteins). Both enzyme levels are increased since the cells which produce them are being destroyed allowing the enzymes to escape into the blood.
- ◆ Chronic pancreatitis can be diagnosed by measuring the level of pancreatic enzymes in the intestine. In all cases the levels of pancreatic enzymes are lower than in normal patients.

Digestive System in Place



Digestive System Displaced



Enzymes as analytical reagents

Enzymes are ideal tools for use in biochemical analysis in both industry and medicine. They have revolutionised many clinical tests which aim to detect the presence of certain compounds in biological fluids (blood, plasma, urine etc) and are widely used in hospitals and laboratories. They are also used in industrial processes e.g. monitoring the production of ethanol and other materials in fermenters. Their two main advantages over other analytical reagents are:

Enzymes have a high specificity: They will only react with one specific substrate, even if that substrate is found in a complex mixture.

Enzymes have a high sensitivity: they can catalyse reactions even when the substrate is present in very low concentrations.

Enzymes are used in three main types of system

Diagnostic test strips: are plastic strips containing a pad with an immobilised enzyme(s) (see below for discussion of immobilised enzymes). These give a semi-quantitative result via a graded colour change. These are very cheap but can only be used once.

Enzyme electrodes or 'biosensors': contain an immobilised enzyme, a transducer and a tiny electric circuit. The enzyme catalysed reaction produces a product which generates an electric current in the transducer. The current is proportional to the amount of product (and hence substrate) present and is converted into a digital read out. These are small and reusable.

Enzyme analytical reactors: are large scale laboratory tools using soluble enzymes linked to dyes which test hundreds of samples per day and give results on the basis of colour changes.

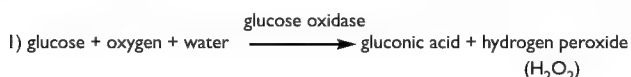
Examples of enzymes used as analytical reagents in medicine and industry include :	
Enzyme(s)	substrate/anylate
Alcohol dehydrogenase:	alcohol
Cholesterol esterase & cholesterol oxidase	cholesterol
Glucose oxidase and peroxidase	glucose
Urease	urea
Penicillinase	penicillin

Using enzymes to test for glucose

One of the most common uses of enzymes as analytical reagents in hospitals, is in tests for blood glucose. The level of glucose in the blood is closely regulated by the pancreatic hormones insulin and glucagon. The normal level is around 90-150mg/100ml of blood. Both high and low levels of glucose are harmful and are useful indicators of disease. In particular, very high levels of glucose in the blood following meals are characteristic of the disease diabetes, as is the presence of glucose in the urine. Glucose levels in blood and urine can be detected using the two enzymes: glucose oxidase and peroxidase.

Diagnostic test strip: for testing glucose levels in urine

The colourless pad on the test strip contains the enzymes glucose oxidase and peroxidase immobilised in a cellulose mat along with a colour reagent (e.g. chromagen). When the pad is dipped into a substance containing glucose the reactions shown below occur. The end point is the blue colour (resulting from the reaction of the hydrogen peroxide with chromagen). The shade of blue reflects the concentration of glucose present and is thus semi-quantitative. The pad takes about 15 seconds to change colour.

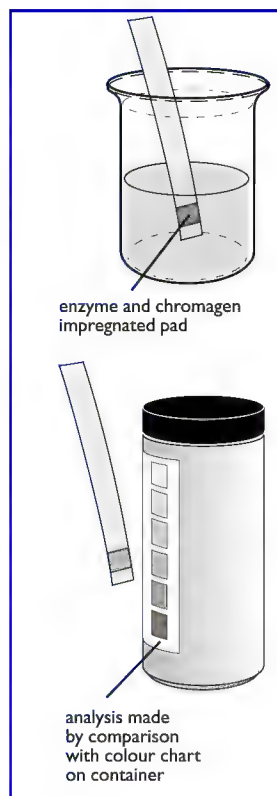


Test strips of this kind are used regularly by diabetics to check their urine for the presence of glucose. A positive result indicates that their blood glucose level was too high. Other simple test strips are available to test blood or urine for a range of compounds including urea (using urease enzyme), and cholesterol (using cholesterol esterase and cholesterol oxidase).

Glucose testing using a biosensor

The biosensor uses the immobilised enzyme glucose oxidase. If a substance containing glucose is placed on the test area of the biosensor the reaction noted above occurs. The gluconic acid produced by the reaction generates an electric current in the transducer which is translated into a digital readout. The read out takes less than 20 seconds to appear and gives a quantitative measure of the amount of glucose present in the sample. This method requires a very small sample: a drop of blood from a pin prick is adequate.

Biosensors, which are easy to use, relatively cheap yet also highly sensitive and specific are commonly used in hospitals to measure the levels of a range of biologically important substances in body fluids.



◆ CHECKPOINT SUMMARY

- ◆ Enzymes are used in three main types of diagnostic system
- ◆ A test strip containing glucose oxidase and peroxidase immobilised in a cellulose mat along with a colour reagent (e.g. chromagen). When the pad is dipped into a substance containing glucose a blue colour develops. The shade of blue reflects the concentration of glucose present and is thus semi-quantitative.
- ◆ Test strips of this kind are used regularly by diabetics to check their urine for the presence of glucose.
- ◆ Enzyme electrodes or 'biosensors': contain an immobilised enzyme, a transducer and a tiny electric circuit which generates an electric current proportional to the amount of substance present and is converted into a digital read out.
- ◆ Enzyme analytical reactors use soluble enzymes linked to dyes which test hundreds of samples per day and give results on the basis of colour changes.

3.9

Drugs are used in the control and treatment of disease

Contents

Betablockers

- ▼ Beta blockers as drugs which can be used to reduce hypertension by binding to receptor molecules.

Antibiotics

- ▼ Antibiotics used to treat bacterial disease by preventing the formation of bacterial cell walls or interfering with the processes of DNA replication and protein synthesis. The importance of the cell wall in preventing osmotic lysis.
- ▼ Bacteriostatic and bactericidal antibiotics and their effect on bacterial populations.

Monoclonal antibodies

- ▼ The use of monoclonal antibodies in enabling the targeting of specific substances and cells.

Introduction

A drug may be defined as any chemical which has an effect on the way in which the body works, although this should be expanded to include the effects of drugs on pathogens in the body. During the twentieth many new drugs have been discovered, whilst others based on naturally occurring substances e.g. in plants have been improved and developed. Successful drugs are those which treat the specific disease problem without causing unwanted side effects.

Three very different types of drug are described below. All of these drugs are specific, affecting only target cells within the body.

BETA BLOCKERS

The treatment of high blood pressure using Beta blockers

High blood pressure is one of the most common disorders in the developed world and is associated with increased risk of death from coronary heart disease, kidney disease and strokes. It refers to the pressure of the blood within arteries and has two components: systolic blood pressure, which is the pressure recorded during contraction of the ventricles and diastolic blood pressure which is the pressure recorded during relaxation of the ventricles. In a healthy young person values around 120mm/Hg over 80mm/Hg would be found. Systolic pressures over 160mm/Hg and diastolic over 95 mm/Hg could indicate high blood pressure, depending on the circumstances.

Beta blockers (or b-adrenergic antagonists) are a group of drugs used for the treatment of high blood pressure and angina (pain in the chest, coming from the heart). They work on both the heart (cardiac) muscle and the smooth muscle in the walls of arteries, and help reduce blood pressure. In particular they reduce cardiac output by slowing heart rate and reducing the force of contraction so that less blood is forced through the vessels per minute.

In the heart, Beta blockers work by binding to Beta-receptor proteins on the cell membranes of muscle cells. They therefore prevent the hormone adrenaline and neurotransmitter noradrenaline from binding to the sites and stimulating the heart to contract more strongly. They thus interfere with both nervous and hormonal stimulation of the heart, and this explains their effect in reducing systolic blood pressure.

Beta blockers also have an effect on the smooth muscle of artery walls, promoting vessel dilation (vasodilation) and hence reducing resistance of vessels to blood flow. This effect explains the reduction in diastolic blood pressure. Beta blockers also reduce anxiety which in turn reduces stress, another factor in high blood pressure.

Antibiotics and the treatment of bacterial infections

An antibiotic is a molecule produced by one microorganism which is capable of destroying or inhibiting the growth of other microorganisms. Most commonly they are chemicals produced naturally by fungi or bacteria which act on other bacteria. Antibiotics work in several ways but are often split into two main categories:

bacteriostatic antibiotics inhibit the growth and replication of bacteria;

bacteriocidal antibiotics kill other bacteria.

Bacteriostatic antibiotics do not generally do lasting damage to bacteria: if they cease to be taken then bacteria can begin to grow and divide once again.

Discovery and use of antibiotics

The discovery of antibiotics was one of the most significant events in medical history. In 1928 Alexander Fleming first noticed that the mould *Penicillium notatum* prevented the growth of bacteria on agar plates. Eleven years later, Ernst Chain and Howard Florey purified the antibiotic penicillin and tested it in mice with bacterial infections. By the mid 1940s several other antibiotics had been found and were being tested on human infections. Antibiotics were found to be extraordinarily effective at killing bacteria and allowed the first effective cures for fatal diseases like syphilis and tuberculosis. They revolutionised the treatment of a wide variety of bacterial infections and have played a major role in preventing illness and death across the world. Many infections which we consider insignificant today, such as streptococcal throat infections and the infections of wounds, caused rapid death before antibiotics were discovered.

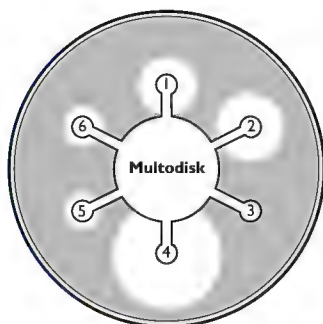
How antibiotics work

Antibiotics work by interfering with one or more of the essential functions of bacterial cells. They do not harm human cells, however, due to the differences in cell structure between bacterial (prokaryotic) and animal (eukaryotic) cells. Antibiotics are usually specific to certain types of bacteria, and thus it is common practice to find out which bacterium is responsible for an infection before attempting to treat it. Broad spectrum antibiotics will work on all or most types of bacteria but can be harmful as they also kill useful bacteria in the gut.

Antibiotics have their effects on bacterial cells in the following ways.

- ▼ Interfering with production of the bacterial cell wall.
- ▼ Limiting or preventing protein synthesis, especially the synthesis of enzymes.
- ▼ Limiting or preventing nucleic acid synthesis and hence cell division.
- ▼ Damaging bacterial cell membranes

Diagram to show action of specific antibiotics on a bacterial culture



- 1 Tetracycline
- 2 Chloraphenol
- 3 Erythromycin
- 4 Sulphafurazole
- 5 Penicillin G
- 6 Streptomycin

Of these modes of action, the prevention of cell wall formation, is probably the most significant. The cell wall of bacteria is made up of long linear carbohydrate polymers called peptidoglycans which have short peptide chains cross-linking them to form a wall with high tensile strength. Antibiotics, including penicillin inhibit the synthesis and formation of these cross links. The result is a weak cell wall which will not protect the bacterium against bursting if it is in a solution that is more dilute (hypotonic) relative to the cell contents. Normally the cell wall resists further movement of water into a turgid cell, but if the wall is defective, water will continue to move into the cell by osmosis until the outward pressure on the wall is so great that it bursts (osmotic lysis).

Antibiotic resistance

When antibiotics were first used they were extremely effective at killing bacteria and hence controlling infections. Over the past fifty years, however, the excessive use of antibiotics has led to some bacteria gaining resistance to the drugs. This means that the drugs are less effective or in some cases completely useless at killing the bacteria. Resistance in the bacteria comes about through natural selection operating on chance genetic mutations in the bacteria. The short generation time and massive reproductive potential of bacteria ensures the rapid proliferation of successful mutant strains.

Two important examples of resistant bacteria (sometimes referred to as 'superbugs') are *Staphylococcus aureus* which is a common cause of infection in hospitals and some types of *Mycobacterium tuberculosis* which causes TB. Rates of illness and death from both of these bacteria are increasing. Health professionals are worried that as more bacteria become resistant to antibiotics, common infections will once again become major causes of death across the world. They are calling for a reduction in the use of the drugs both for treating mild infections, as well as in the farming industry where they are often included in animal feeds.

Monoclonal antibodies

Monoclonal antibodies are antibodies grown in the laboratory from a single B-lymphocyte clone. All the antibodies produced from such a clone are identical and will thus be specific to one antigen. As described previously (section 12.3) they are produced from cells known as hybridomas which are made by fusing a cancer cell (myeloma) with a B-lymphocyte of the desired type. Monoclonal antibodies can theoretically be produced against any antigen, not just against those on the surface of pathogens. Thus whilst they have many potential uses in the control and treatment of disease, they have a wide range of other applications, e.g. in the pregnancy testing kit where monoclonal antibodies detect the presence of the hormone HCG in the urine of a pregnant woman.

Uses of MONOCLONAL ANTIBODIES in the TARGETING of SPECIFIC SUBSTANCES and CELLS

The targeting of specific substances.

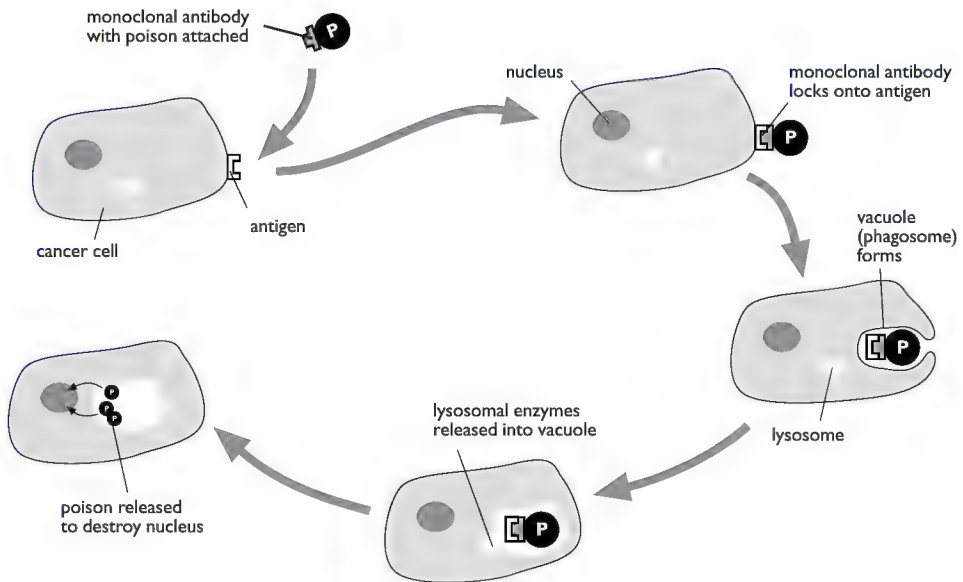
A number of research trials have used monoclonal antibodies to bind to and inactivate cytokines in the body. For example, the cytokine TNF has been implicated in a number of inflammatory disorders and infectious diseases. Anti-TNF monoclonal antibodies have been given to patients suffering from diseases such as rheumatoid arthritis, malaria, and septic shock resulting from bacterial infections. New types of monoclonal antibody are being investigated for treating overdoses with common drugs such as aspirin and paracetamol.

The targeting of specific cells

Diagnosis of cancer. Research has demonstrated the possibility of using monoclonal antibodies to detect the presence of cancer cells whose surface antigens differ from those of normal human cells. Monoclonal antibodies with fluorescent or radioactive tags could be injected into the body and used to detect cancer cells. Screening could then locate the position of the cancer cells by detecting the presence of the marker tag. Cancers could therefore be detected earlier improving the chances of treatment and recovery.

Treatment of cancer. This involves attaching toxic drugs or radioactive isotopes to monoclonal antibodies specific to cancer cells. The antibodies could then attach to the target cells destroy them, leaving non-cancerous cells unharmed.

Diagram showing mechanics of a 'magic bullet'



Diagnosis of infectious disease. The specificity of monoclonal antibodies make them ideal tools to use in the diagnosis of infectious diseases. Such tests are currently in use for HIV and the Herpes viruses. They work on the principle that if a pathogen is present in a blood sample then antigens on its surface will bind to specific monoclonal antibodies attached to a plastic plate. A series of stages involving further antibodies and an enzyme catalysed colour change allows detection of a positive result. This is known as an ELISA (enzyme linked immunosorbent assay). If a pathogen (or, in some cases, even a strain of pathogen) other than that being tested for is present it will not be detected as it will have a different type of antigen.

◆ CHECKPOINT SUMMARY

- ◆ A drug may be defined as any chemical which has an effect on the way in which the body works, and on any pathogens in the body.
- ◆ Beta blockers (or b-adrenergic antagonists) are a group of drugs used for the treatment of high blood pressure and angina (pain in the chest, coming from the heart).
- ◆ They reduce cardiac output by slowing heart rate and reducing the force of contraction so that less blood is forced through the vessels per minute.
- ◆ An antibiotic is a molecule produced by one microorganism which is capable of destroying or inhibiting the growth of another microorganism. Most commonly they are produced by fungi or bacteria to act on other bacteria.
- ◆ Antibiotics work in several ways but there are two main categories: bacteriostatic antibiotics inhibit the growth and replication of bacteria; bacteriocidal antibiotics kill other bacteria.
- ◆ Antibiotics use one or more of the four main targets listed below to attack bacterial cells:
 - ◆ Interfering with formation of the bacterial cell wall.
 - ◆ Limiting or preventing protein synthesis, especially the synthesis of enzymes.
 - ◆ Limiting or preventing nucleic acid synthesis and hence cell division.
 - ◆ Damaging bacterial cell membranes
- ◆ Monoclonal antibodies are antibodies grown in the laboratory from a single B-lymphocyte clone. All the antibodies produced from such a clone are identical and will thus be specific to one antigen.
- ◆ Monoclonal antibodies have many potential uses in the control and treatment of disease, and other applications: e.g. in the pregnancy testing kit where monoclonal antibodies detect the presence of the hormone HCG in the urine of a pregnant woman.
- ◆ Monoclonal antibodies can detect the presence of cancer cells whose surface antigens differ from those of normal human cells.
- ◆ Monoclonal antibodies with fluorescent or radioactive tags could be injected into the body and used to detect cancer cells.
- ◆ Toxic drugs or radioactive isotopes can be attached to monoclonal antibodies specific to cancer cells. These then attach to the target cells destroy them, leaving non-cancerous cells unharmed.
- ◆ The specificity of monoclonal antibodies make them ideal tools in the diagnosis of infectious diseases. Such tests are currently in use for HIV and the Herpes viruses.

Topic Guide



- 1.1 The cell as the basic unit of structure in prokaryotic and eukaryotic organisms**
- 1.2 The electron microscope and the technique of cell fractionation to study ultrastructure**
- 1.3 The properties of plasma membranes and the passage of substances through them**
- 1.4 Large molecules in the structure and functioning of cells**
- 1.5 Enzymes as proteins which control biochemical reactions in cells**
- 1.6 Tissues and organs**
- 1.7 The blood system and transport**
- 1.8 The heart and its role in the circulation**

Prokaryotic cells

Session:

▼ Respiration is located in mesosomes which are infoldings of the cell surface membrane, sharing

▼ Prokaryotic cells may form colonies but never multicellular organisms/tissues.

Much of the original work on the function of DNA was done on prokaryotic cells, write a paragraph suggesting difficulties in extrapolating the findings of this work to eukaryotic cells.

Notes:

Molecules, Cells & Systems

1.1 The cell as the basic unit of structure in prokaryotic and eukaryotic organisms

Eukaryotic cells

Topic Guide

Session: _____

Check List

- ▼ Eukaryotes do have a true membrane bound nucleus containing chromosomes of DNA and histone proteins, and membrane bound organelles as described previously.
- ▼ Eukaryotic cells typically form tissues in multicellular organisms.
- ▼ Cell differentiation and specialisation prevent the easy definition of typical animal and plant cells.
- ▼ 'Typical' cells would be those considered to show features common to the vast majority of cells in animals or plants
- ▼ A squamous (pavement) epithelium cell from the lining of the mouth could be considered as a 'typical' animal cell.
- ▼ A mesophyll cell from a leaf could be considered as a 'typical' plant cell.
- ▼ Under the best light microscope both the typical animal cell and the typical plant cell can be seen to possess a cell surface membrane, cytoplasm, nucleus with nucleolus, mitochondria, endoplasmic reticulum, and Golgi body.
- ▼ In addition the typical plant cell can be seen to possess a cell wall around the cell surface membrane, a large central vacuole surrounded by the tonoplast membrane, and chloroplasts.

Student Activity

Notes: _____

Materials

- Microscopes
- Slides of squamous epithelium
- Slides TS Leaf showing palisade cells
- OHT Photomicrograph of animal cells
- OHT Photomicrograph of plant cells

Procedure

Make LP and HP drawings of the plant and animal cells. Using the OHTs for guidance.

Note the major differences:

- a) in what is actually seen;
- b) what is known from theory studies.

Explain the reasons for differences between (a) and (b)

Set Work

Write a brief justification of 'interpretation' in Biological drawing, e.g. drawing the plant cell wall as a double line (which is not actually seen down the typical lab microscope).

Molecules, Cells & Systems

10.1 The cell as the basic unit of structure in prokaryotic and eukaryotic organisms

Eukaryotic cells

Topic Guide

Session: _____

Check List

- ▼ If the specimen can be observed with the naked eye without the need for magnification the calculation of the linear magnification of any drawing of it is straightforward - being the number of times any linear dimension of the drawing is greater than that of the specimen.
- ▼ Magnifications of images of slides of biological material are often given in terms relating to the magnification of the microscope image, e.g. $\times 100$ and $\times 400$.
- ▼ However reproductions of the image as seen down a microscope introduce another layer of magnification which is often overlooked.
- ▼ To calculate the linear magnification of drawings of microscope images of specimens it is necessary to know how many times the drawing is larger than the actual specimen.
- ▼ The actual size of the specimen is measured with an eye piece graticule and stage micrometer.

Student Activity

Materials

- Microscopes
- Material for the preparation of temporary mounts
- Slides of TS Leaf
- Practical Work Sheet:
The preparation of temporary mounts, the use of simple staining techniques and the estimation of size
- ☐ The use of graticule and stage micrometers

Procedure

- ☐ OHT aids understanding of the use of an eye piece graticule and stage micrometer.

Work out the linear magnification of a drawing that you make of a structure seen under the microscope. Explain all your steps fully.

Set work

Calculate the magnification of the area of a circle with a diameter of 2 cm, when the diameter is doubled (linear magnification of $\times 2$), and trebled (linear magnification of $\times 3$).

Try to work out why microscopic animals appear to move so fast when seen moving under the microscope.

Notes

Molecules, Cells & Systems

1.1 The cell as the basic unit of structure in prokaryotic and eukaryotic organisms

Eukaryotic cells

Topic Guide

Session:..

Check List

- ▼ If the specimen can be observed with the naked eye without the need for magnification the calculation of the linear magnification of any drawing of it is straightforward - being the number of times any linear dimension of the drawing is greater than that of the specimen.
- ▼ Magnifications of images of slides of biological material are often given in terms relating to the magnification of the microscope image, e.g. $\times 100$ and $\times 400$.
- ▼ However reproductions of the image as seen down a microscope introduce another layer of magnification which is often overlooked.
- ▼ To calculate the linear magnification of drawings of microscope images of specimens it is necessary to know how many times the drawing is larger than the actual specimen.
- ▼ The actual size of the specimen is measured with an eye piece graticule and stage micrometer.

Student Activity

Materials

- Microscopes
- Material for the preparation of temporary mounts
- Slides of TS Leaf
- Practical Work Sheet:

The preparation of temporary mounts, the use of simple staining techniques and the estimation of size

- ☐ The use of graticule and stage micrometers

Procedure

- ☐ OHT aids understanding of the use of an eye piece graticule and stage micrometer.

Work out the linear magnification of a drawing that you make of a structure seen under the microscope. Explain all your steps fully.

Set work

Calculate the magnification of the area of a circle with a diameter of 2 cm, when the diameter is doubled (linear magnification of $\times 2$), and trebled (linear magnification of $\times 3$).

Try to work out why microscopic animals appear to move so fast when seen moving under the microscope.

Biology Advanced Subsidiary

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Notes

Molecules, Cells & Systems

1.2 The electron microscope and the technique of cell fractionation may be used to study ultrastructure

Electron microscopy

Topic Guide

Session: _____

Check List

- ▼ Resolution is the ability to see detail.
- ▼ The higher the resolution the more of the detail present can be seen.
- ▼ Magnification is enlarging in size.
- ▼ Increasing magnification only reveals extra detail if the detail can be resolved.
- ▼ Resolving power of light microscope limited by the wavelength of light.
- ▼ Put simply some detail is so small that it cannot be 'hit' by light, and therefore cannot be seen.
- ▼ Endless magnification of the image produced by a light microscope yields no extra information.
- ▼ Wavelengths of electron beams much shorter than that of light and give the electron microscope much greater resolving power than the light microscope.
- ▼ Therefore extra magnification up to $\times 250\,000$ yields an equivalent amount of detail.

Student Activity

Materials

- Microscope
- Prepared slide of TS dicotyledonous leaf
- ☐ OHT Magnification and Resolution.
- ☐ OHT Electron micrograph

Procedure

Set up slide of TS Leaf showing xylem on high power

Although this may be the first sight of TS dicotyledonous leaf, it can clearly be seen to be made up of cells.

Even under the highest power ($\times 400$) very little detail will be seen within the cells.

- ☐ OHT Magnification and Resolution.
- ☐ OHT Electron micrograph

Measure the size (diameter) of a mitochondrion on the electron micrograph and calculate the actual size by dividing by the magnification.

Set work

1. Distinguish between the terms 'magnification' and 'resolution'.
2. Why can more detail be seen using a beam of electrons than using visible light?

Notes: _____

Molecules, Cells & Systems

1.2 The electron microscope and the technique of cell fractionation may be used to study ultrastructure

Cell ultrastructure

Topic Guide

Session: _____

Check List

- ▼ The 'typical' animal cell under the electron microscope (EM) is the mammalian liver cell, as it contains a representative selection of those organelles found in animal cells.
- ▼ The 'typical' plant cell under the EM is a leaf mesophyll cell.
- ▼ The risk of distortion and the production of artefacts is greater with the EM as a result of the total dehydration, very thin sectioning, staining, and high temperatures generated.
- ▼ It has been argued for example that the rough or granular endoplasmic reticulum is an artefact caused by the cracking and splitting of the cytoplasm pushing the ribosomes to the edge.
- ▼ Mitochondria are rod-shaped organelles but appear oval in cross section. Chloroplasts are oval in structure and appear oval in cross section
- ▼ Lysosomes cannot be identified solely on appearance, the activity of digestive enzymes must be demonstrated by other means.
- ▼ The rough and smooth endoplasmic reticula are not related in structure or function, and are not joined. Typically there is less smooth endoplasmic reticulum in plant cells.
- ▼ Controversy surrounds the identification of centrioles in plant cells.

Student Activity

Materials

- A selection of e.m.s showing whole cells or parts of cells, both animal and plant.
- T.E.M. image of a whole cell (unlabelled, for homework)
- OHT Ultrasound scan of fetus in uterus

Procedure

- OHT Ultrasound scan of fetus in uterus demonstrates that something imaged in an unfamiliar way requires practice to interpret.

Examine the electronmicrographs.

Is it plant or animal? Look at the junction between adjacent cells. Is there a cell wall? Any large spaces?. Look to identify the larger things first, e.g. nucleus, mitochondria, chloroplasts.

Discuss clues which point to particular functions e.g. lots of mitochondria, cilia, microvilli etc.

Set work

Label the T.E.M. of an animal cell before the next lesson.

Notes:

Session:

10.2 The electron microscope and the technique of cell fractionation may be used to study ultrastructure

Cell ultrastructure

- ▼ Functions of organelles determined by differential centrifugation, and biochemical investigation of samples from each 'organelle band'.
- ▼ Centrifuged 'organelle bands' not completely 'pure'.
- ▼ Lysosomes poorly identifiable in electron micrographs, identification almost entirely on functional criteria.
- ▼ Radioactive tracers can locate functions in specific organelles.
- ▼ Cells specialised for particular functions show organelles developed in similar way, e.g. active voluntary muscle, and secretory cells, have

- ▼ Smooth endoplasmic reticulum associated with a wide range of metabolic functions suggesting that its identification as a specific organelle is unlikely.
- ▼ Existence of many ribosomes arranged in chains (polysomes) suggests protein synthesis (translation of mRNA) in action.
- ▼ There are still major problems in correlating specific functions with clearly identifiable organelles.
- ▼ Function of chloroplasts easily established with the use of the light microscope, i.e. recognising cell fraction that carries out photosynthesis.

Notes:

- A selection of electronmicrographs showing whole cells or parts of cells, both animal and plant.
- T.E.M. image of a whole cell

Revise the identification of all organelles and membrane systems.

Construct a table of organelles and their function.

Explain the structure and function of a mitochondrion in relation to its surface area to volume ratio.

Molecules, Cells & Systems

1.2 The electron microscope and the technique of cell fractionation may be used to study ultrastructure

Cell ultrastructure

Topic Guide

Session: _____

Check List

- ▼ Prokaryotic cells (bacteria) lack a true nucleus and membrane bound organelles.
- ▼ The DNA is located in a central strand, and as circular plasmids in the rest of the cytoplasm.
- ▼ The cell wall is not cellulose as in plant cells.
- ▼ Respiration is located in mesosomes which are infoldings of the cell surface membrane, sharing the principle of increased surface area of membranes with true mitochondria.
- ▼ The origin of mitochondria and chloroplasts as mutualistic prokaryotes with eukaryote cells, explains their absence in prokaryotes.
- ▼ Ribosomes are present in both prokaryotes and eukaryotes.
- ▼ Prokaryotic cells may form colonies but never multicellular organisms/tissues.

Student Activity

Materials

■ Microscope and slides of bacteria

□ OHT Structure of bacterium

Procedure

Observe the prepared slides of bacteria, and calculate the size, either by approximation or the use of the stage micrometer and eye piece graticule.

Observe □ OHT Structure of bacterium and estimate the magnification needed to reveal such detail.

Set work

Explain the relationship between the structure of an individual bacterium and the type of colony that they form.

Notes.

Molecules, Cells & Systems

1.2 The electron microscope and the technique of cell fractionation may be used to study ultrastructure

Cell fractionation

Topic Guide

Session: _____

Check List

- ▼ Isolation of subcellular components starts with a mechanical disruption of the cells to produce subcellular fragments.
- ▼ A pH buffer and sucrose solution is added to prevent disruption of the organelles, and the temperature is lowered to prevent autodigestion by enzymes, and decomposition by bacteria.
- ▼ The suspension of cell fragments is spun at high speeds in an ultracentrifuge.
- ▼ The force that is exerted upon the suspension forces the organelles downwards to the bottom of the tube according to their mass and density.
- ▼ The nuclei are the first to be forced down at about 1000G (1000 x the force of gravity).
- ▼ The supernatant liquid can be separated off and respun at higher speeds.
- ▼ Mitochondria spin down at about 3000G.
- ▼ Smaller organelles like the ribosomes require much higher forces to separate them out.
- ▼ Isolated samples are never pure, and can be further purified by recentrifuging in a liquid medium of graded density, with the greatest density at the bottom.
- ▼ Organelles separate at a level in the liquid corresponding to their own density.

Student Activity

Materials

- Centrifuge
- Blender
- Ice
- Iodine in potassium iodide solution
- Microscope and slides

Procedure

Macerate some leaves (spinach) in ice cold 0.5 M sucrose in the blender, filter through some muslin, and spin at low speed in the centrifuge for 10 minutes. This should separate down cell walls and starch grains.

Pour off supernatant and re-centrifuge at high speed for 10 minutes to separate out chloroplasts.

Observe samples of the fractions under the light microscope.

Stain both fractions with iodine in potassium iodide solution to test for the presence of starch.

Set work

Write a short account of your findings.

Notes: _____

Molecules, Cells & Systems

1.3 The properties of plasma membranes and the passage of substances through them

Plasma membranes

Topic Guide

Session: _____

Check List

- ▼ All cell membranes are thought to share the same structure except the inner membrane of mitochondria.
- ▼ This is based on a lipid bilayer formed from phospholipids aligning themselves with their hydrophobic poles next to each other within the membrane, and hydrophilic poles facing outwards.
- ▼ This fluid arrangement is stabilised by the presence of another lipid - cholesterol.
- ▼ Various proteins are embedded within this lipid bilayer.
- ▼ Extrinsic proteins are found on the surface of the membrane.
- ▼ Intrinsic proteins have one end embedded in the lipid bilayer and the other protruding out of the external surface of the membrane.
- ▼ Transmembrane proteins span the lipid bilayer and create aqueous channels of pores through which water soluble substances can pass by diffusion.
- ▼ Short polysaccharide chains attached to proteins (glycoproteins) and to fats (glycolipids) protrude from the external surface and act as cell recognition sites (receptors or antigens).
- ▼ The patchwork arrangement of all these types of molecules in the lipid bilayer is the origin of the description 'mosaic'.

Student Activity

Materials

- ☐ OHT Phospholipids and the bilayer
- ☐ OHT The fluid mosaic model

Procedure

☐ OHT Phospholipids and the bilayer explains the structure of cell membranes, building up the fluid mosaic model from the phospholipid bilayer. Use this OHT as a back transparency for drawing in first cholesterol (slows down lateral movement of phospholipids and also makes the membrane less permeable), then the three types of protein, extrinsic, intrinsic, and transmembrane. Finally draw in glycoproteins and glycolipids.

☐ OHT summarises the final structure.

Place some well washed identical slices of beetroot (washed until no more pigment is released) in beakers of water at a range of temperatures from 0°C to 100°C; and in a range of dilutions of detergent, and observe the release of pigment from the slices. Use a colorimeter if available.

Set work

List all the structures in plant, animal and bacterial cells which include membranes in their structure.

Tabulate the results from the experiment with beetroot slices, and comment on the results.

Notes:

Molecules, Cells & Systems

1.3 The properties of plasma membranes and the passage of substances through them

Diffusion

Topic Guide

Session: _____

Check List

- ▼ The cell surface membrane defines the limits of the cell.
- ▼ It receives 'messages' by providing receptor sites for hormones, cell recognition of 'self' and 'non-self', and act as antigens with which antibodies can bind.
- ▼ It acts as a partially permeable membrane regulating the transport of substances across the membrane.
- ▼ Internal membrane systems provide a surface for attachment of organelles and enzymes, and compartmentalise specialised functions within the cell.
- ▼ Passive diffusion occurs across membranes down diffusion gradients from high concentration to low concentration. Fat soluble substances diffuse faster than non-fat soluble substances which must pass through the aqueous pores.
- ▼ The speed of these diffusing particles is only increased by an increase in temperature, which increases their kinetic energy. In addition to the effect of temperature on diffusion, the amount of a substance that diffuses in a given time (rate of diffusion) is dependent upon the difference in its concentration in two separated regions (the concentration gradient), the surface area of the separating boundary or exchange surface, and the distance separating them i.e. the thickness of the exchange surface; a relationship expressed by Fick's law.

Rate of diffusion is proportional to:

difference in concentration x surface area: divided by the thickness of the exchange surface

Student Activity

Notes: _____

Materials

- Gelatine and methylene blue
- 'Jelly' mould or equivalent
- OHT Diffusion and facilitated diffusion

Procedure

Make up different sized cubes of gelatine coloured with methylene blue, wash well to remove any surface dye, and submerge in clear water.

Observe the diffusion of dye from the cubes.

Use a colorimeter or colour chart to try to quantify rate of diffusion of dye out of cubes with respect to surface area to volume ratio.

Set work

Use an example from the human body to illustrate Fick's law.

Topic Guide

Session: _____

Molecules, Cells & Systems

1.3 The properties of plasma membranes and the passage of substances through them

Water potential

Active transport and facilitated diffusion

Endo- and exocytosis

Check List

- ▼ Osmosis is the special case of the diffusion of water from regions of higher water potential to regions of lower water potential across a partially permeable membrane which is more permeable to water than to dissolved solutes. (If the membrane was fully permeable the solutes would diffuse down their diffusion gradient and the water would not move.)
- ▼ At room temperature and pressure pure water has the highest water potential which is given the value of zero. Solutions have a water potential of less than zero (negative).
- ▼ Facilitated diffusion occurs via specialised carriers in the membranes which reduce the resistance to the diffusion of these substances.
- ▼ Active transport involves energy in the form of ATP from respiration to pump ions across the cell surface membrane via carriers against their diffusion gradient.
- ▼ Mass or bulk transport of fluids into and out of the cell is achieved by vacuoles fusing with, or being pinched off from, the cell surface membrane respectively.

Student Activity

Materials

- Range of plant food storage material
- 1M sucrose solution
- Methylene blue
- Practical Work Sheet:
The movement of water down water potential gradients through partially permeable membranes.

☐ OHT Water potential

Procedure

Investigate the water potential of a range of plant material: potato chips, beetroot (which allows for a check on any losses from damaged cells), onion rings, banana slices of different ripeness.

Measure changes in dimension and/or mass.

An alternative approach is to investigate any alterations of density of bathing solution by colouring with methylene blue and releasing a drop of this carefully at mid-depth into a beaker of the original solution, if water has entered the cells by osmosis the bathing solution will have become more concentrated, and will sink when introduced into original solution. If the bathing solution has gained water from the cells it will be less dense than the original and rise when introduced into the original.

Osmosis and diffusion can be studied in the same investigation if solutions of urea are used. Initially plant material loses water to concentrated urea solution and shrinks and loses mass, but after a

period urea diffuses into the plant material which recovers size and mass. A puzzling and interesting phenomenon to observe.

Set work

Comment critically on the the relative merits of using changes in dimensions or mass to detect losses and gains of water.

Notes: _____

Biological molecules

Session:

- ▼ Most of the important biological molecules are 'organic', which means that they are made up from carbon compounds
- ▼ Not all compounds containing carbon are organic. Exceptions include carbon dioxide and carbonates).
- ▼ Organic biomolecules include carbohydrates, proteins, lipids and nucleic acids,
- ▼ Individual molecules (monomers) can join to form dimers of just two monomers, or longer chains (polymers) of repeating monomers.
- ▼ Polymers are very large molecules, built up by reactions called condensation reactions in which

- ▼ Bonds formed by condensation reactions may be broken down by the reverse process, hydrolysis, which involves the addition of a molecule of water for each bond broken.
- ▼ Condensation and hydrolysis reactions are catalysed by enzymes in the living cell.
- ▼ Under laboratory conditions, in the absence of a catalyst, hydrolysis requires extreme conditions of temperature and pH .

Tabulate the results from the biochemical tests.

Notes:

Molecules, Cells & Systems

1.4 Large molecules in the structure and functioning of cells

Carbohydrates

Topic Guide

Session: _____

Check List

- ▼ Simple formula for glucose - $C_6H_{12}O_6$
- ▼ Forms straight chain in dry powder form.
- ▼ Forms ring structure in solution.
- ▼ alpha and beta glucose are isomers differing in the 3D arrangement of their atoms.
- ▼ Monosaccharides (glucose, fructose and galactose) combine together with the elimination of water to form glycosidic bonds - a condensation reaction.
- ▼ Glycosidic bonds are broken by the addition of the elements of water across the bond - a hydrolysis reaction.
- ▼ Starch is a mixture of amylose (unbranched chains) and amylopectin (branched chains).
- ▼ Both being long chains (polymers) of alpha glucose molecules.
- ▼ When combined the alpha glucose molecules have their oxygen bridges on the same side of the chain, hydrogen bonds form between them and cause the chain to coil into a helix.
- ▼ Starch molecules are relatively insoluble in water.
- ▼ Cellulose is a polymer of beta glucose which have their oxygen bridges on alternate sides of the chain and so cannot form a helix.
- ▼ Instead they form hydrogen bonds with other adjacent chains to make flat ribbons which form cellulose fibres.

Student Activity

Materials

- Molecular model of alpha and beta glucose.
- Gravy powder, bunsen, gauze, and beaker with water
- Small tasting samples of glucose, fructose, sucrose in powdered form on sterile filter papers
- OHT Monosaccharides and disaccharides
- OHT Polysaccharides

Procedure

- OHT Monosaccharides and disaccharides recaps structure of alpha and beta glucose

Observe molecular models of glucose.

- OHT Polysaccharides

To illustrate the principle of starch hydration, dissolve the gravy powder in a small volume of water, then heat gently in the beaker (safety glasses etc). Describe how and when it thickens, then discuss why this happens.

'Blind' tasting of fructose, glucose and sucrose.

Put the substances in order of sweetness. Discuss how properties can vary with small molecular differences. (Caution - do not use galactose. Some people are intolerant)

Set work

Make molecular diagrams to show the structure of alpha and beta glucose, maltose, starch and cellulose.

Notes: _____

Proteins

Session:

- ▼ All amino acids share a common structure consisting of a hydrogen atom, an amino group (NH_2), a carboxyl acid group (COOH), and a 'R' group, all attached to a carbon atom.
- ▼ The 'R' group can range from a single hydrogen atom to a long complex chain. Thus the simplest amino acid has the formula of $\text{CH}_2\text{NH}_2\text{COOH}$.
- ▼ Amino acids combine in a condensation reactions which form a peptide bond.
- ▼ There are about 20 different types of amino acids found in proteins of living organisms.
- ▼ Proteins are polymers of up to 2000 amino acids joined by peptide bonds (polypeptides)
- ▼ The combination of the 20 different types of amino acids gives rise to as many as 100 000 different

- ▼ The sequence of amino acids in the polypeptide chain determines the primary structure of proteins.
- ▼ Hydrogen bonds between adjacent NH_2 and COOH groups result in the secondary structure of either alpha helix or beta sheet. Both of these structures may occur in the same protein.
- ▼ Bonds that form between the 'R' groups determine the tertiary structure.
- ▼ Combination or association of two or more polypeptide chains determines the quaternary structure of a protein.
- ▼ Haemoglobin has a quaternary structure involving four polypeptide chains and four iron containing haem groups.

Materials

- Molecular models of two amino acids, one with an 'R' group of just one hydrogen atom, and one with a long chain.
- Popper beads.
- OHT Amino acid structure and peptide bond
- OHT Secondary and tertiary structures of proteins
- OHT Structure of haemoglobin

- OHTs revise structures.

In what ways are they different.

Notes:

Molecules, Cells & Systems

1.4 Large molecules in the structure and functioning of cells

Lipids

Topic Guide

Session: _____

Check List

- ▼ Triglycerides are composed of three (tri) fatty acids combined by condensation reactions to form ester bonds with glycerol.
- ▼ Fatty acids have long hydrocarbon chains and a carboxyl group (COOH).
- ▼ Saturated fatty acids (and hence saturated fats) have no double bonds in the hydrocarbon chain which is 'saturated' with hydrogen.
- ▼ Unsaturated fatty acids have one double bond.
- ▼ Polyunsaturated fatty acids have two or more double bonds.
- ▼ Lipids include fats, oils, waxes, phospholipids and steroids.
- ▼ Fats (triglycerides) are energy rich storage products in plants and animals.
- ▼ Phospholipids are formed from triglycerides by the replacement of one fatty acid by a phosphate group.
- ▼ Phospholipids form the structural basis of biological membranes by forming a bilayer.
- ▼ The bilayer is formed with their hydrophobic poles adjacent and their hydrophilic poles in contact with the aqueous phase.

Student Activity

Materials

- Range of fats, oils and waxes
- OHT Structure of triglyceride
- OHT Phospholipids and the bilayer

Procedure

Observe the range of fats, oils and waxes.

Tabulate the location and functions of fats, oils, waxes, phospholipids, cholesterol and steroids in living organisms.

Set work

Make notes to explain the structural differences between saturated and non-saturated fats

Notes: _____

Chromatography

Session:

- ▼ Paper Chromatography is used to separate small traces of materials from a mixture.
- ▼ A concentrated spot of the mixture is placed near one end of a piece of chromatography (or filter) paper. The end of the paper is then dipped in a solvent solution such as ethanol and water.
- ▼ As the solvent front moves up the paper, so substances dissolved in it are carried along.
- ▼ The rate of movement for each substance depends upon its solubility in the solvent relative to water, so different substances move different distances depending upon how easily they enter the water phase associated with the cellulose fibres of the paper.
- ▼ The paper is removed from the solvent before it reaches the top, then the solvent front is marked and the paper, now called a chromatogram, is allowed to dry.

- ▼ By comparing the distance travelled by the solvent with the distance travelled by each component of the mixture, a comparative value, called the Rf (relative front) value can be obtained.
- ▼ The separation may be enhanced by performing a second run of solvent, this time at right angles to the first, producing a two way chromatogram.
- ▼ A slightly more sophisticated and faster version of chromatography, this technique makes use of a sheet of glass coated with an absorbent gel (e.g silica or starch). The glass plate is dipped into a solvent just like the paper in the method described above.
- ▼ Examples of the use of chromatography in biology include the separation of photosynthetic pigments from plant leaf extracts

- Filter paper
- Chloroplast 'pellet' from centrifugation of spinach leaves
- Solvent mixture of acetone and petroleum ether
- Tubes
- Practical Work Sheet:

Chromatography

Carry out the simple separation of chlorophyll pigments using a safe and supervised procedure.

Practical Work Sheet 4: Chromatography contains more complete instructions.

Mount your simple chromatography paper strip and label the different pigments identified.

Notes:

Enzyme Action

Session:

- ▼ All enzymes are protein in nature, and fall into the group of 'globular' proteins with a complex 3D structure.
- ▼ Enzymes are catalysts which speed up the rate of reaction of much larger amounts of reactants without themselves being used up in the process.
- ▼ Unique amongst catalysts they are denatured by extreme conditions of pH and temperature etc.
- ▼ Their activity depends upon their complex 3D

▼ Disruption of the 3D shape accounts for denaturation.

Find out the name of an inorganic catalyst, the reaction that it catalyses, and the conditions necessary for the reaction to proceed.

Notes:

Factors which affect enzyme activity

Session:

▼ Similar effects are seen with temperature, except low temperatures do not denature enzymes. The rate of enzyme catalysed reactions, like all chemical reactions, are reduced with low temperatures.

▼ Rates of formation of end-product(s) are proportional to the rate of reaction.

Notes:

Explain the relationship shown by the two curves.

Molecules, Cells & Systems

1.6 Tissues and Organs

Epithelial tissue

Blood

Topic Guide

Session: _____

Check List

- ▼ In multicellular organisms cells are organised into tissues. A tissue being an association of similar cells specialised and coordinated to a particular function. Some functions being more specialised than others.
- ▼ Examples of tissues in mammals include squamous epithelium covering surfaces e.g. in the kidney tubule and the alveoli of the lungs, ciliated epithelium lining the trachea and bronchi, cerebrospinal canal, and oviduct.
- ▼ Some tissues consist of only one type of cell. e.g. ciliated epithelium, whilst others consist of several variants of similar types of cells.
- ▼ Cells in the same tissue develop from the same original cell type, even though the fully differentiated cells may appear very different.
- ▼ Different tissues become 'organised' into organs specialised to particular functions, e.g. a lung is an organ and consists of squamous epithelium, ciliated epithelium, connective tissue, blood vessels etc.
- ▼ Blood consists of fluid plasma with red blood cells, white blood cells (or white cells) and blood platelets.
- ▼ Plasma has 10% materials in suspension and solution.
- ▼ Plasma proteins wide variety of functions and act to buffer changes in pH.
- ▼ Materials carried in solution not strictly part of the plasma.
- ▼ Red blood cells lose nucleus in development and become biconcave corpuscles of haemoglobin.
- ▼ Large surface area to volume ratio individually due to biconcave shape, and huge total surface area due to small size (6-8 μm) and huge numbers.
- ▼ Red blood cell volume 30-50% of total blood volume.
- ▼ Males higher blood count/haemoglobin than females.
- ▼ White cells of which there are several types form basis of immune system (see section 5.2.6)
- ▼ Platelets are fragments of white cells.

Student Activity

Materials

- Slide of stained blood smear
- Practical Work Sheet:
Microscopic examination of tissues and organs
- ☐ OHT Blood components
- ☐ OHT Diagram showing relationship between blood and lymph

Procedure

Observe slide of stained blood smear with aid of OHT showing structure and function of red cells, lymphocytes, phagocytes and platelets.

Red blood cells are amongst the smallest things seen in this course and the light and sub-stage condenser must be carefully adjusted otherwise it is difficult to see them.

White cells are widely scattered and typically stained blue, especially the nucleus. It may be difficult to differentiate between the different types.

Platelets may just be seen under HP as tiny granules.

Notes: _____

Molecules, Cells & Systems

1.6 Tissues and Organs

Blood vessels

Topic Guide

Session: _____

Check List

- ▼ Elasticity of arteries important in smoothing flow of cardiac output. Expansions and contractions is felt as the pulse.
- ▼ Finer branches and arterioles main site of generation of resistance to blood flow.
- ▼ Capillary beds supply all tissues providing large surface areas for exchanges by diffusion and short diffusion distances.
- ▼ Capillaries have large total cross sectional surface areas. Therefore resistance to flow, blood pressure and rate of flow all drop in capillaries.
- ▼ Diameter of capillary same as red blood corpuscles thus forcing them to pass through capillaries in single file, bringing all close to tissues.
- ▼ Plasma minus proteins is exuded through thin walls to bathe tissues directly.
- ▼ Thin walls and large lumen of veins offer little resistance to flow of blood back to heart, and allow ease of massage by surrounding muscles.
- ▼ Pocket valves ensure one way flow back to heart.
- ▼ Venous blood is under low pressure but the flow is fast enough to ensure return of blood to heart at same rate as blood flow in the arteries.

Student Activity

Materials

- 2mm (ring) sections cut from the aorta and vena cava of a sheep/pig's heart in saline
- 40 cm length of fishing line.
- Assorted weights (10 to 100g) and hook for their suspension.
- Retort stand and 2 clamps.
- Ruler

Procedure

Investigation into stretch and recoil of arteries and veins.

Set up a retort stand with the section of artery or vein suspended by the fishing line to a clamp. A second piece of fishing line is used to suspend different weights from the vessel. A ruler is positioned and clamped so as to enable any stretching of the vessel to be measured. The exercise consists of adding weights to the line. Measure the distance stretched as a 10g weight is added and record it as a percentage of the original vessel length, then take off the weight and measure how far the vessel recoils. Record this also as a percentage of the original vessel length. Repeat,

increasing the weight by 10g each time. Observe the differences in stretch and recoil properties of arteries veins. Suggest a reason for the differences.

Set work

Make a table to compare the structure and functions of arteries, veins and capillaries.

Notes.

Molecules, Cells & Systems

1.7 The Blood System and Transport

Circulation

Topic Guide

Session: _____

Check List

- ▼ Closed double mammalian circulation more efficient than open single circulation.
- ▼ Open circulation has blood returning to heart in large open blood spaces (haemocoel), slow flow and low pressure.
- ▼ Compensated for in insects by tracheal system delivering oxygen gas (in air) directly to tissues.
- ▼ Single circulation blood only passes through heart once on each complete circulation of the body.
- ▼ Means that blood passes through two sets of capillaries on each circulation, respiratory surface and body tissues.
- ▼ Large resistance to overcome before blood returns to heart, resulting in slow flow and low pressure in veins which are typically large blood sinuses to lower resistance to flow.
- ▼ Closed systems blood almost entirely restricted to vessels: arteries, arterioles, capillaries, venules, veins.
- ▼ Tissue fluid exuded to bathe tissues directly.
- ▼ Double circulation blood passes through heart twice on each circulation.
- ▼ Pressure relatively low to lungs.
- ▼ Pressure raised for systemic circulation to body giving high pressure and fast flow.

Student Activity

Materials

- ☐ OHT Mammalian heart
- ☐ OHT Closed double circulation diagram

Procedure

- ☐ OHT Mammalian heart
- ☐ OHT Closed double circulation. Explain the advantages of this figure of a double circulation (it only occurs in birds and mammals - the 'warm blooded' vertebrates).

Reptiles have incomplete division of ventricles.

Relate this diagram to the structure of the heart using the ☐ OHT.

Set work

Write 10 lines on 'Why the human heart can be considered as two separate pumps.'

Notes:

Molecules, Cells & Systems

1.7 The Blood System and Transport

Capillaries

Topic Guide

Session: _____

Check List

- ▼ The capillaries form a fine network which penetrate all tissues.
- ▼ They have the smallest diameter of all blood vessels, about 0.007 mm or 7 micrometres (μm), the same as that of the red blood cells, forcing red cells to move slowly through the capillaries in single file, ensuring a short diffusion distance between the corpuscles and the tissues.
- ▼ Capillary walls are just one cell thick, which further aids exchanges by diffusion between the blood and the tissues.
- ▼ The huge number of capillaries provide a very large surface area across which these exchanges occur.
- ▼ Tissue fluid is formed when plasma, minus most of its proteins, is pushed out under pressure through the capillary walls.
- ▼ Most of the tissue fluid is reabsorbed back into the blood capillaries.
- ▼ Tissue fluid bathes each cell and is the medium for the exchange of materials between the individual cells and the blood system.
- ▼ Tissue fluid which is not reabsorbed by the blood capillaries enters the lymphatic capillaries and becomes lymph.
- ▼ The blind-ended lymphatic capillaries lead into wider lymph vessels which are similar in structure to the veins, having thin walls with pocket valves.
- ▼ Contraction of skeletal muscles massages the vessels, and the valves ensure unidirectional flow. This aids the draining of tissue fluids.
- ▼ At intervals in the system are swellings of reticular tissue, called lymph nodes, which play an important role in the defence of the body against infection (ref 3HB 12.3).
- ▼ The lymph is returned to the circulation in the anterior venae cavae.

Student Activity

Materials

- Microscopes
- Slides TS alveoli
- OHT Capillary network

Procedure

□ OHT Capillary network demonstrates the principle of capillary networks, but diagrams cannot come close to illustrating the size and complexity of capillary beds.

To get a more realistic idea (and introduce the next topic) view the microscope slides of TS alveoli.

Alveoli are surrounded by some of the richest capillary networks in the body.

Set work

Write a paragraph on why the formation of tissue fluid is reduced in the lungs.

Notes: _____

Topic Guide

Session: _____

Molecules, Cells & Systems

1.7 The Blood System and Transport

Lung function

The gross structure of the human gas-exchange system

The exchange of respiratory gases in the lungs

Check List

- ▼ Gas exchange occurs over large surface area of alveoli which is a product of their relatively small size and large number.
- ▼ Diffusion gradients of oxygen and carbon dioxide dependent upon partial pressures of gases on either side of diffusion surface.
- ▼ Diffusion surface composed of thin squamous epithelium ridged around the sandwiched capillaries.
- ▼ Surface water an inevitable consequence of a permeable surface exposed to air, decreases diffusion of oxygen more than the diffusion of carbon dioxide.
- ▼ Air in alveoli remains fairly constant in composition independent of breathing cycle.
- ▼ Diffusion gradients maintained by circulating blood and ventilation of the lungs.
- ▼ As demand for oxygen increases so does steepness of diffusion gradients and speed of circulation thus meeting extra demand.

Student Activity

Materials

- Microscopes
- Slides TS alveoli
- OHT Diagram of alveoli and associated capillaries

Procedure

□ Use OHT showing the structure of alveoli and associated capillaries to help interpretation of microscope slide of TS alveoli. A copy of the OHT diagram could be annotated as the lesson progresses. Compile a list of factors which affect the rate at which oxygen diffuses from the alveolar space to combine with haemoglobin in a red cell in the capillary. You should think of the following: relative concentration of oxygen (partial pressures), distance to be travelled, surface area for exchange, speed of blood flow, ventilation rate.

Consider the diffusion of oxygen in a theoretical situation where the blood and air are static. The rate would depend entirely on the surface area, the concentration gradient, the distance and the degree of oxygenation of haemoglobin. Now consider what happens as the air and the blood move. Remember that during exercise there may be a large increase in oxygen uptake per minute. Annotate a copy of the diagram by way of making notes on this lesson.

Set work

In the disease emphysema the alveoli run together to form larger air sacs. Explain, the effect this has on the surface area of the lung for gas exchange, illustrating your answer with simple diagrams and calculations.

Notes: _____

Molecules, Cells & Systems

1.7 The Blood System and Transport

Ventilation

Topic Guide

Session: _____

Check List

- ▼ For efficient gaseous exchange at the alveolar surface, air must be moved in and out of the lungs in the process called ventilation.
- ▼ Inspiration (breathing in) - the external intercostal muscles contract, pulling the ribs upwards and outwards, the diaphragm contracts, moving downwards, the volume of the thorax increases, the pressure in the thorax decreases below atmospheric pressure causing air to enter, inflating the lungs.
- ▼ Expiration (breathing out) depends upon the elasticity of the tissues of the lungs and thorax. The respiratory muscles relax, the thorax and lungs recoil as a result of their elasticity and the events of inspiration are reversed.
- ▼ At sea level the composition of inspired air is relatively constant, but the composition of expired air varies with the activity of the individual.
- ▼ More oxygen is consumed and carbon dioxide produced as activity increases.
- ▼ Normally, respiratory movements are involuntary, being controlled by rhythmic discharges of nerve impulses from the respiratory control centres in the medulla region of the brain.
- ▼ From the inspiratory control centre impulses travel down the phrenic nerves to the diaphragm, and down the intercostal nerves to the external intercostal muscles causing them to contract and bring about inspiration.
- ▼ At the end of inspiration, stretch receptors in the bronchioles send impulses to the inspiratory control centre in the medulla, which is switched off, and the diaphragm and external intercostal muscles relax. The elasticity of the the tissues of the lungs and thorax leads to expiration as they recoil.

Student Activity

Notes: _____

Materials

- Practical Work Sheet:

Investigating the effects of exercise on the composition of expired air

- OHT Simple apparatus for the measurement of carbon dioxide levels in expired air.

Procedure

The investigation of carbon dioxide levels in expired air at differing levels of activity can be used as a pilot study for the practical requirement of the effects of exercise on the body in later in this module.

Set Work

Write up your results from the investigation.

Session:

1.8 The Heart and its role in the circulation

Pressure and volume changes and associated valve movements during the cardiac cycle

- ▼ The events of a complete filling and emptying of the heart are known as the cardiac cycle.
- ▼ It is a continuous cycle the start point of which can be taken when heart is empty and all valves shut.
- ▼ Atria fill and internal pressure rises forcing bicuspid and tricuspid valves open.
- ▼ Ventricles fill so that whole heart full.
- ▼ Atria contract (atrial systole) so that ventricles overfilled and stretched.

- ▼ Ventricles contract with a force proportional to their degree of stretch (ventricular systole).
- ▼ Blood forced up shutting bicuspid and tricuspid valves (first heart sound) and opening pocket valves as blood forced up pulmonary artery and aorta.
- ▼ Ventricles relax (ventricular diastole) blood falls back in pulmonary artery and aorta shutting pocket valves (second heart sound).

Notes:

- Blood pressure measuring device
- Stethoscope

Procedure

Demonstrate the measurement of systolic and diastolic blood pressure (on yourself) Explain the process and significance.

Stand back to a wall and raise arm to level with heart. At this point veins in hand and arm should still be visible (if visible in the first place). Mark this position. Get an assistant to raise the arm until veins 'collapse' mark this new position. Note the distance (d) between the two marks in cms. Calculate the venous blood pressure from:

Set work

Record your pulse rate before rising each morning and just before going to bed each evening for a week, and plot as a graph of pulse rate against time.

Session: _____

1.8 The Heart and its role in the circulation

Myogenic stimulation of the heart

Roles of sinoatrial node, atrioventricular node and bundle of His

- ▼ Cardiac muscle is myogenic.
- ▼ Rate modified by sinu-atrial node (pacemaker) in wall of right atrium.
- ▼ Waves of electrical activity spread rapidly down through cardiac muscle of atria.
- ▼ Prevented from passing directly into ventricles by barrier of connective tissue, stimulus picked up by atrio-ventricular node.
- ▼ Bundle of His composed of Purkyne tissue transmits stimulus to base of ventricles from

- ▼ Detected by electrodes on the skin as an ECG.
- ▼ Pacemaker influenced by sympathetic (faster) and parasympathetic (slower) nervous systems, and the hormone adrenaline.
- ▼ All influences on heart rate coordinate heart rate and therefore blood supply to body (cardiac output) with demands of body.

Materials

- ## Procedure

Relate the ECGs to the events illustrated on the OHT.

Set work

Artificial pacemakers re-establish a fixed rhythm in those suffering erratic heart beats. Explain the effect on daily activities of having an artificial pacemaker fitted.

Notes:

Effects of Exercise

Session:

▼ The blood supply to the brain and heart remains relatively stable during exercise, but increases dramatically to the working muscles from about 20 to 88%

Notes:

Topic Guide



- 2.1 Isolation of enzymes from microorganisms and applications in biotechnological processes**
- 2.2 The transfer of genetic information from cell to cell during division**
- 2.3 Genes and the genetic code**
- 2.4 Gene technology and its applications**
- 2.5 Forensic examination of blood**
- 2.6 Cultivated plants and the manipulation of their environment to increase productivity**
- 2.7 Biotechnology and the manipulation of reproduction in humans and domestic animals**

Applied Biology

2.1 Isolation of enzymes from microorganisms and applications in biotechnological processes

Isolation of enzymes

Topic Guide

Session: _____

Check List

- ▼ Intracellular enzymes are produced inside cells and work inside cells. Extracellular enzymes are produced inside cells but released from cells to work outside.
- ▼ Growth of large numbers of microorganisms in fermenters for the commercial production of enzymes requires a suitable nutrient medium in which to grow, suitable environmental conditions, and absence of competition from other microorganisms (ie. sterile conditions).
- ▼ Nutrient requirements vary between species but all microorganisms require simple organic sources of carbon and nitrogen (such as simple sugars and amino acids), and a range of vitamins and minerals dissolved in water.
- ▼ Given optimal conditions microorganism growth will follow a characteristic pattern. It will begin slowly and then grow exponentially.
- ▼ If the bacteria are left alone their growth rate will level off and then decline until it crashes to zero as nutrients and oxygen have been used up.
- ▼ Alternatively, if, the bacteria are given more nutrients and oxygen and other conditions are kept optimal they will continue to grow at a maximum rate.
- ▼ It is essential that the fermenter and all pipes leading to and from it are sterile before microorganisms are introduced. This also applies to all substances added to the fermenter during fermentation. Such sterile conditions are described as aseptic.
- ▼ The majority of enzymes used in industrial processes are extracellular. This reflects the fact that isolation and purification of intracellular enzymes is usually difficult and expensive.
- ▼ Extracellular enzymes are secreted from microorganisms into the culture medium and can usually be isolated from this medium with relative ease.
- ▼ Intracellular enzymes can only be obtained by damaging the cells in which they are produced. Cells may be disrupted mechanically or physically by procedures such as freezing-thawing, osmotic shock, the use of high temperatures, high pHs or digestion by other enzymes.
- ▼ Once the cells have been broken up in this way filtration or centrifugation is used to remove the cell debris and ultrafiltration or chromatography to purify the desired enzyme.

Student Activity

Materials

□ OHT Fermenter

Procedure

Use □ OHT Fermenter as a focal point for a review of the commercial production of enzymes.

Set work

Explain why most commercially exploited enzymes are extracellular.

Notes: _____

Applied Biology

2.1 Isolation of enzymes from microorganisms and applications in biotechnological processes

Application of enzymes in biotechnological processes

Topic Guide

Session: _____

Check List

- ▼ Enzymes are ideal tools for use in biochemical analysis in both industry and medicine. They have a high specificity and will only react with one specific substrate, even if that substrate is found in a complex mixture.
- ▼ Enzymes have a high sensitivity: they can catalyse reactions even when the substrate is present in very low concentrations.
- ▼ Enzymes are used in three main types of system: diagnostic test strips: enzyme electrodes or 'biosensors': enzyme analytical reactors.
- ▼ Industrial processes require a high degree of thermostability (heat tolerance).
- ▼ The higher the temperature the lower the chance of contamination with micro-organisms, the faster the rate of reaction, the less viscous the substrate and the higher the solubility of reactants.
- ▼ Immobilised enzymes are enzymes that have been attached to another (insoluble) material in some way and hence prevented from moving freely within the substrate mixture. They may be physically immobilised within a system by being attached to a fixed membrane or gel made of a range of substances including silica, starch, and cellulose.
- ▼ Immobilised enzymes may be more easily added to and removed from reactors to allow the rate of reaction to be more carefully controlled.
- ▼ Immobilised enzymes can lead to much greater production efficiency. Primarily they can be used in a continuous enzyme reactor where substrate is constantly added and product constantly produced without the need to add or extract enzyme.
- ▼ Immobilised enzymes tend to be more stable (better able to resist alteration to shape and activity.) In particular they are less likely to be inactivated or denatured by high temperatures (they are more thermostable), changes in pH, or presence of other chemicals.
- ▼ Immobilised enzymes can also be used for longer before their activity decreases and are less likely to lose activity during periods of storage.
- ▼ A disadvantage of immobilising enzymes is that the rate of reaction may be slower because the enzyme has reduced kinetic energy and is often partitioned from the substrate by a membrane through which the substrate must diffuse.

Student Activity

Notes: _____

Materials

- Practical Work Sheet:

The action of Immobilised Lactase enzyme on Lactose in milk

- OHT Immobilisation of enzymes

Procedure

Make a flow chart of the High Fructose Corn Syrup process.

Set work

Write an account of one use of enzymes as biosensors.

Applied Biology

2.2 The transfer of genetic information from cell to cell during division

Mitosis The Cell Cycle

Topic Guide

Session: _____

Check List

- ▼ Mitosis is nuclear division in which the daughter nuclei have the same chromosome number as the parent nucleus, typically diploid nuclei producing diploid daughter nuclei.
- ▼ The daughter nuclei are therefore genetically identical to the nucleus of the cell that produced them (except for any mutations that may occur).
- ▼ Mitotic cell division is seen in growth, repair and asexual reproduction.
- ▼ As a result of mitosis in the growth of an organism from the fertilised egg all cells therefore are genetically identical.
- ▼ Similarly, offspring of asexual reproduction lack genetic variation.
- ▼ The production of genetically identical cells retains the full set of genetic information needed for the replacement of any cells in repair processes, and in the asexual reproduction of organisms it allows for the rapid exploitation of optimum conditions where any variation would be disadvantageous.
- ▼ The DNA replicates in interphase so that in the first stage of mitosis (prophase) the chromosomes appear as double structures of two chromatids (daughter chromosomes) joined at the centromere.
- ▼ The nuclear spindle apparatus(NSA) forms from the dissolution of the nuclear membrane and the centrioles replicate and migrate to either pole of the NSA.
- ▼ The centromeres align on the equator of the NSA (metaphase).
- ▼ The centromeres divide and the chromatids are moved to the opposite poles (anaphase).
- ▼ Two new daughter nuclei are formed (telophase).
- ▼ Mitosis represents one phase of the cell cycle of division
- ▼ The cell cycle is completed by the formation of two new cells by division of the cytoplasm.

Student Activity

Materials

- Microscopes
- Slides of root tip squashes
- Practical Work Sheet:

Preparation of an onion root tip squash to show stages in mitosis

- ☐ OHT Root tip squash
- ☐ OHT Normal Human Karyotype

Procedure

Observe slides of root tip squashes.

Use ☐ OHT Root tip squash for guidance.

Make drawings of prophase, metaphase, anaphase and telophase nuclei.

Look for similarities between the root tip cells and the human nucleus.

Set work

Determine at what stage in mitosis the photograph of the human nucleus and chromosomes was taken, and from what type of cell. Explain your reasons.

Notes:

22 The transfer of genetic information from cell to cell during division

Meiosis

Topic Guide

Session:

Check List

- ▼ Each gamete of a sexually reproducing organism contains one set of chromosomes (haploid).
- ▼ When two such gametes fuse in fertilisation they produce a zygote with two sets of chromosomes (diploid).
- ▼ Chromosomes in a diploid nucleus thus occur in pairs, each member of a pair carrying the same genes in the same relative positions (homologous chromosomes).
- ▼ Slight variations (alleles) of a gene at a particular locus can arise by mutation, so that an organism can either have an identical pair of genes at a locus (homozygous) or a pair that differs slightly (heterozygous).
- ▼ Pairs of sex determining chromosomes are only partially homologous e.g. the human Y chromosome has a short non-homologous arm which carries the male determining genes.
- ▼ The production of haploid gametes from a diploid cell requires a reduction division (meiosis) in which the chromosome number is halved from diploid to haploid.
- ▼ The production of haploid gametes ensures the maintenance of the diploid chromosome number in subsequent generations.

Student Activity

Materials

- Handout of Human Karyotype analysed into homologous pairs
- OHT Human Karyotype analysed into homologous pairs

Procedure

Examine the pairs of homologous chromosomes of the Human Karyotype. Note that before the development of staining techniques that revealed differences in dark staining bands, it was not possible to pair up the chromosomes into homologous pairs with any confidence.

Set work

Explain why chromosome studies are carried out on squashes of cells and not sections.

Notes:

Applied Biology

23 Genes and the genetic code

DNA as genetic material

Structure of nucleic acids

Replication of DNA

Topic Guide

Session: _____

Check List

- ▼ The nucleus of cells contains mostly DNA and protein.
- ▼ The DNA content doubles in cells just prior to dividing.
- ▼ It was also found that, in the formation of gametes (sperms and eggs), the amount of DNA halved. This evidence underlines its central role in inheritance.
- ▼ Now it has become possible to analyse the molecular structure of DNA, to unravel the chemical code it contains, and to transfer pieces of this code between one organism and another in the process known as genetic engineering.
- ▼ DNA and RNA are long chain polynucleotide molecules with repeating sub-units of nucleotides.
- ▼ Nucleotides consist of a 5 carbon sugar, a phosphate, and an organic base.
- ▼ In DNA the sugar is deoxyribose and the organic base can be one of adenine, thymine, cytosine and guanine.
- ▼ In RNA the sugar is ribose and the organic base can be one of adenine, uracil, cytosine and guanine.
- ▼ The DNA molecule is a double stranded helix with the two strands held together by hydrogen bonds between complementary base pairs adenine-thymine and cytosine-guanine.
- ▼ RNA is a single stranded helix with base pairs of adenine-uracil and cytosine-guanine.
- ▼ There are three types of RNA - messenger, transfer and ribosomal.
- ▼ DNA carries out semi-conservative replication in which each strand of the original molecule acts as a template for the construction of a new one, so that each new double stranded molecule of DNA consists of one original strand and a new complementary strand.

Student Activity

Materials

- ☐ OHT Structure of DNA
- ☐ OHT Replication of DNA

Procedure

Use OHTs to revise and discuss the structure and replication of DNA

Set work

Write a paragraph to explain what is meant by semi-conservative replication of DNA.

Notes: _____

Applied Biology

23 Genes and the genetic code

Protein synthesis

Topic Guide

Session: _____

Check List

- ▼ The sequence of bases (and hence nucleotides) on one strand of the DNA molecule carries the genetic code for the synthesis of proteins and hence ultimately living cells.
- ▼ Three bases (a triplet codon) code for one amino acid.
- ▼ There are 64 possible combinations of any three from four bases, but only 20 amino acids to be coded for.
- ▼ Amino acids are coded for by more than one triplet codon and the code is said to be 'redundant' or 'degenerate'.
- ▼ A gene is a sequence of nucleotides of a DNA molecule which codes for a polypeptide.
- ▼ A gene for a polypeptide has a specific 'start' codon which thus sets the order of all the subsequent triplet codons
- ▼ Thus the code is non-overlapping i.e. a base can only be 'used' in one triplet codon.
- ▼ Only a fraction of the nuclear DNA contains genes coding for proteins, the rest consists of so-called 'junk' DNA the function of which is not understood.
- ▼ Nuclear DNA with the genetic code never leaves the nucleus in eukaryotic cells.
- ▼ The genetic code for a polypeptide (a gene) is copied (transcribed) into mRNA which leaves the nucleus and associates with the ribosomes.
- ▼ The ribosomes 'read' the genetic message on the mRNA and tRNA deliver amino acids in the sequence determined by the code.
- ▼ There are 20 different types of tRNA, each specific to a particular amino acid.
- ▼ Each tRNA has a specific binding site at which the specific amino acid combines and an anti-codon which matches the specific codon for that particular amino acid.
- ▼ The amino acids combine by means of peptide bonds to give a polypeptide chain.
- ▼ The polypeptides subsequently form proteins.
- ▼ As enzymes are proteins their synthesis is controlled by DNA.

Student Activity

Materials

- ☐ OHTs showing base sequence / gene code
- ☐ OHT Transcription and translation
- ☐ OHT Table of amino acid codons

Procedure

Use OHTs to revise and discuss the genetic code
Can be used to revise translation and transcription.

Set work

Draw out a random sequence of bases of a strand of DNA and annotate it to demonstrate the non-overlapping nature of the genetic code.

Draw out the complementary strand of DNA to the one that you have drawn and annotate to illustrate how only one strand can carry the genetic information.

DNA replication, transcription, and mRNA translation all require enzymes to catalyse their reactions. Explain the particular problem that this represents in terms of protein synthesis.

Notes: _____

Recombinant DNA

Session:

- ▼ Isolation of the gene to be manipulated is the first step
- ▼ It is easier to find and isolate the appropriate mRNA which has been transcribed (copied) from the relevant DNA, than it is to try and isolate the particular length of DNA itself.
- ▼ The enzyme reverse transcriptase can then be used to make a copy of the DNA from the mRNA.
- ▼ Plasmids (circular DNA) are isolated from bacteria.
- ▼ Plasmids are cut open by restriction enzymes and the length of DNA is inserted and the plasmid resealed by ligase enzymes.
- ▼ The genetically modified plasmids are then

- ▼ The bacteria multiply and produce the protein coded for by the inserted DNA.
- ▼ To identify those bacteria which have successfully taken up the modified plasmids marker genes are incorporated along with the required gene.
- ▼ Marker genes include those for antibiotic resistance so that the culture can be flooded with antibiotic and only the bacteria with the marker gene will survive.
- ▼ Two early successes with these techniques were the synthesis of human insulin for the treatment of diabetics, and blood clotting factor VIII for the treatment of haemophiliacs.

Notes:

Applied Biology

2.5 Forensic examination of blood

Principles of immunology

Topic Guide

Session: _____

Check List

- ▼ When pathogens enter the body tissues there are two branches of the immune system in operation within the body which co-operate with each other to some extent.
- ▼ Non-specific immunity which is not targeted at specific types of pathogen but at foreign material in general. Involves phagocytosis and inflammation and is immediate.
- ▼ Specific immunity which uses B and T lymphocytes to target specific pathogens. Such responses are more complex and are often delayed.
- ▼ B-lymphocytes are concerned with Humoral immunity, involving antibodies.
- ▼ T-lymphocytes are concerned with Cell-Mediated immunity with the direct killing of infected cells.
- ▼ Immunology is the study of the mechanisms by which the B and T lymphocytes recognise and destroy foreign material which enters the body.
- ▼ In all cases, foreign or 'non-self' material is distinguished from 'self' material by the presence of 'non-self' antigens.
- ▼ Antigens are defined as molecules which, when foreign to an individual, stimulate an immune response by lymphocytes in the body.
- ▼ There are many thousands of specific types of B-lymphocyte and each is capable of recognising only one specific antigen. This allows specific antibodies to be produced in response to a vast range of antigens.
- ▼ Antibodies are a type of protein molecule known as immunoglobulins which are secreted by activated B-lymphocytes when a specific antigen is encountered.
- ▼ The antibodies attach to the antigens on the foreign material and lead to the destruction of it in one of three ways:
- ▼ Memory cells remain in the lymph nodes and the circulation. If a subsequent infection with the same pathogen occurs then some of these will turn into plasma cells and start producing antibodies. This is the basis of natural active immunity to diseases and underlies the practice of vaccination (artificial active immunity).
- ▼ T-lymphocytes do not produce antibodies but work directly on infected cells. They may either lyse the cells directly by secreting enzymes or may interact with other branches of the immune system to effect their killing.
- ▼ ABO blood groups are examples of antigens on the surfaces of red blood cells. Group A individuals have A antigens on the surface of their red blood cells, Group B people have B antigens, Group AB, both A and B antigens and group O have no antigens.
- ▼ The blood plasma of individuals of each of the four blood groups also differs, Group A will have antibodies against group B antigens (anti-B antibodies), and a person of Group B will possess anti-A antibodies. Since an AB individual has both A and B antigens on their red cells they will have neither anti-A or anti-B antibodies in their plasma. A group O person possesses both anti-A and anti-B antibodies.

Student Activity

Materials

□ OHT Immunology a) b)

Procedure

Survey duration of and intervals between infections in the group

Set Work

Name a non-fatal disease that can only be caught once in a lifetime, and one that can be caught several times. Write a paragraph explaining the reason for this.

Applied Biology

2.5 Forensic examination of blood

Genetic fingerprinting

Polymerase chain reaction

Topic Guide

Session: _____

C3.2: C3.3

Check List

- ▼ Genetic fingerprinting, also known as DNA profiling relies on the extensive genetic variation which exists in the 'non-coding' DNA which lies between genes or in the introns of genes and makes up 95% of the genome.
- ▼ Much of this non-coding DNA contains repeated sequences of sections of DNA between 15 and 100 bases long. Within such regions known as VNTRs (variable number of tandem repeats) or minisatellites the number of repeats of a sequence is highly variable between individuals. Analysis of only a small number of VNTR regions can produce a unique profile.
- ▼ Creating a genetic fingerprint from the DNA of an individual involves a number of stages:
- ▼ Extraction of the DNA from cells (may be white blood cells, sperm cells in semen, hair cells etc).
- ▼ Amplification of the DNA using polymerase chain reaction (PCR).
- ▼ Use of specific restriction enzymes to cut the DNA at specific sites either side of VNTR region.
- ▼ Use of gel electrophoresis to separate the DNA fragments up on the basis of their size (i.e on the number of repeats).
- ▼ Southern blotting and use of radioactive DNA probe to locate the fragments of DNA.
- ▼ Autoradiography to create an image of the DNA pattern for interpretation.
- ▼ Genetic fingerprinting is used in forensic investigations where DNA collected at the scene of a crime can be analysed and compared to the genetic fingerprints of known individuals or to fingerprints prepared from unknown individuals.
- ▼ For reliable comparisons several VNTR sites must be compared. It is estimated that if one single locus probe is used the same pattern of bands will occur in 1 person out of 100. But when 2 are used the number jumps to 1 in 10 000. With several probes the chances of two sets of DNA matching are so low that the test becomes highly specific in identifying individuals.

Student Activity

Materials

- ☐ OHT DNA fingerprinting technique
- ☐ OHT DNA fingerprints

Procedure

- ☐ OHT DNA fingerprinting technique
- ☐ OHT DNA fingerprints

review this rather technical topic.

Set Work

Write a paragraph on why evidence from the scene of a crime cannot be based entirely on DNA fingerprinting of samples found at the scene.

Notes: _____

Applied Biology

26 Cultivated plants and the manipulation of their environment to increase productivity

Adaptations of cereals

Topic Guide

Session: _____

Check List

- ▼ Cereals are seed crops derived from the family of plants known as the Graminae, or grasses. They provide the main energy component (staple food) of most human diets; rice in the Far East, wheat and barley in the Middle East, *Sorghum* and millet in Africa, and maize in Central and South America.
- ▼ Rice can be grown on dry land like wheat, even in semi-temperate areas but the yield increases up to three times when it is grown in paddy fields in tropical or sub-tropical temperatures. Over half the world production is in irrigated soil.
- ▼ For most of the growing period the rice plants are partially submerged but the land is drained just before harvest so that the dry seed can be collected efficiently.
- ▼ Rice roots have an unusually high tolerance to anaerobic conditions in flooded soils, and their seeds can germinate in oxygen starved soils.
- ▼ In common with other water adapted plants they also develop an interconnecting system of large air spaces linking the roots with the leaves and other parts above water. This specialised tissue is called aerenchyma and it allows the flooded roots to breathe.
- ▼ Maize thrives in hot climates with well watered soils and is adapted to photosynthesise successfully at light intensities and temperatures higher than most plants can tolerate and with a reduced supply of carbon dioxide.
- ▼ Maize and some other tropical plants, notably sugar cane and *Sorghum*, have evolved a mechanism referred to as C4 photosynthesis which overcomes the problem of photorespiration by increasing the concentration of stored carbon dioxide in their leaves. This gives them a higher light saturation point, and therefore a higher productivity.
- ▼ Like maize, *Sorghum* is a C4 plant, specialised to maximise photosynthetic yield in high light intensities and hot climates. Compared to maize, however, *Sorghum* is able to tolerate dry conditions, requiring 20% less water to produce equivalent yields of dry matter, and is able to grow in more hostile climates than any other cereal crop.
- ▼ *Sorghum* being specially adapted to tolerate dry conditions is a xerophyte. The adaptations (xerophytic features) centre on ways of reducing water loss whilst retaining photosynthetic activity.
- ▼ *Sorghum* is similar to maize in its general structure but its leaves are more specialised for water conservation. They are coated with a white waxy layer giving them extra protection from desiccation and the stomata are situated in sunken pits in order to minimise unnecessary water loss whilst allowing gas exchange. In conditions of water stress, the leaves reduce their exposed surface area by becoming erect and curling inwards.
- ▼ *Sorghum* has a very extensive rooting system with twice as many lateral branches as that of maize. Its roots also extend deep enough to extract water in drought conditions.

Student Activity

Materials

- Rice grains
- Maize cobs
- Corn flakes
- OHT Maize

Procedure

Relate the crops to familiar everyday food products

Set Work

Chicken fed on white rice grains develop a vitamin deficiency disease, whilst chickens fed on whole rice grains remain healthy. Write a paragraph explaining these observations.

Applied Biology

26 Cultivated plants and the manipulation of their environment to increase productivity

Controlling the abiotic environment

Topic Guide

Session: _____

Check List

- ▼ Productivity is measured as the gain in plant dry mass per square metre of land surface. This reflects the relative rates of photosynthesis and respiration.
- ▼ The abiotic environment includes light intensity, temperature, and carbon dioxide concentration.
- ▼ Increasing light intensity increases the rate of photosynthesis up to a certain point, the light saturation point, past which there are no further gains as the result of light stimulated photorespiration.
- ▼ Temperature, like carbon dioxide and light, can be a limiting factor in photosynthesis, but it is more accurately regarded as an indirect influence, because above 15-20°C, it affects the rate of respiration more strongly than the rate of photosynthesis.
- ▼ The most important factor affecting productivity is the average night temperature. 'Dark respiration' uses up twice as much assimilated carbohydrate at 25°C than at 15°C, for example, so cool nights may promote better growth.
- ▼ Most temperate crops give their best production between 10 -15°C, but the optimum for other regions varies from 5 °C in some arctic species to 30°C for a tropical crop like maize.
- ▼ The quantity of carbon dioxide in the atmosphere remains relatively constant at 0.04%, but local concentrations around a crop can range from 0.015 - 0.1%, and under natural conditions in daylight carbon dioxide is the rate regulating (rate limiting) factor for photosynthesis.
- ▼ By maintaining all the limiting environmental and nutritional factors at their optimum level in controlled glasshouses it is possible to achieve a high yield in a small space and a short time period.
- ▼ An understanding of the various environmental factors controlling plant growth has led to the development of soil-free or hydroponic plant culture systems.
- ▼ Where day length influences flowering as in the case of chrysanthemums it is controlled by the use of artificial light and/or partial shading.
- ▼ The optimum temperature for tomatoes and lettuces is 18-22°C. It is important to vary the day and night temperature by 6-12°C in order to minimise dark respiration. The temperature directly affects humidity so fine mist sprays are linked to the same control system.
- ▼ Glasshouse plants, being confined to an enclosed space, quickly use up the available carbon dioxide. Where heating is the norm, a hydrocarbon burner may be used to supply carbon dioxide. Alternatively, dry ice or gas cylinders may be used.

Student Activity

Materials

- ☐ OHT Effects of light intensity, temperature and carbon dioxide on rate of photosynthesis and productivity

Procedure

Concentrate on the idea of rate regulating (limiting) factors on photosynthesis and productivity.

Set Work

Write a paragraph on the disadvantages of glasshouse crop production.

Notes: _____

Applied Biology

Increase productivity

Fertilisers

Topic Guide

Session: _____

Check List

- ▼ Harvesting removes nutrients from the soil by preventing the nutrients in the crop from returning to the soil as a result of decomposition.
- ▼ Crop productivity is affected by the availability of inorganic nutrients in the soil, which are supplemented by the addition of fertilisers.
- ▼ The mineral nutrients most frequently applied to soils as fertiliser are nitrogen, phosphorus and potassium. They are often combined in what is known as an NPK mixture. The relative amounts of each component and the timing of the application can influence both the development and yield of a crop.
- ▼ Nitrogen is the most important limiting nutrient because without it, plants cannot make proteins.
- ▼ Up to 80% of phosphate fertiliser, normally applied in the form of 'super phosphate' (P_2O_5), can be wasted by becoming fixed in compounds not available to plants, so it is sometimes introduced with the seed as it is sown, a practice called banding ensuring that the nutrient is available where the roots actually grow.
- ▼ Potassium is absorbed in large quantities by plant roots.
- ▼ Organic fertilisers should be seen as complementary to, rather than an alternative to, the inorganic (chemical) fertilisers described above. The main source of organic nutrients is animal waste, either in solid form mixed with bedding materials (farmyard manure) or as liquid or semi-liquid (slurry).
- ▼ Organic fertilisers improve soil structure by binding small particles together and retaining water.
- ▼ Modern intensive animal farming methods tend to use less bedding and the dung is stored in tanks in liquid or semi-liquid form. Liquid manures are potentially very hazardous to the environment. They have a high biological oxygen demand (BOD) causing eutrophication and, far from improving soil structure, they tend to create anaerobic conditions by flooding the soil air spaces.
- ▼ Green manure is the term used to describe the practice of growing and ploughing into the soil a green (non-seed) crop such as white mustard.
- ▼ The main nutrient which leaches out of the soil in any quantity to pollute rivers and lakes is nitrate. The nitrate acts as a fertiliser in the aquatic ecosystem causing 'super feeding' or eutrophication.
- ▼ Eutrophication causes unnaturally rapid growth of plants, occasionally forming algal blooms on the surface of the water.
- ▼ The increase in plant material, over a period of time leads to increased amounts of dead organic matter which become food for aerobic bacteria. Bacteria have a high biological oxygen demand (BOD) and consequently starve the other oxygen uses of essential supplies. The highest oxygen users, notably fish and certain insect larvae suffer population decline and extinction.
- ▼ In extreme cases conditions become anaerobic and the ecosystem collapses.

Student Activity

Materials

- ☐ OHT Eutrophication and oxygen sag in flowing water

Procedure

Use ☐ OHT Eutrophication and oxygen sag in flowing water to revise the topic.

Set Work

Make a list of the advantages and disadvantages of organic and inorganic fertilisers.

Applied Biology

26 Cultivation of plants to increase productivity

Pesticides

Topic Guide

Session: _____

Check List

- ▼ Weeds compete with crops for water, light and mineral nutrients and generally show better tolerance to limited supplies. They have high rates of photosynthesis, and rapid development, so they out compete the crop plant.
- ▼ Weeds tend to be the first colonisers of cultivated ground, more resistant than other plants to environmental extremes, and more tolerant of changing conditions. Some secrete toxic or growth retardant substances called allelochemicals to fight off the competition.
- ▼ The closer the requirements of crop and weed, the greater the competition, and the more difficult to control the weed, e.g. when the weed and crop belong to the same plant family.
- ▼ If the relationship is very close there is a further danger of hybridisation between weed and crop to produce an even stronger competitor.
- ▼ Harvest yields are affected most if the growth and reproductive stages of the weed coincide with those of the crop.
- ▼ The success of insects as competitors for human food may be explained by the hard exoskeletal material, chitin, which can be fashioned into many different feeding accessories that no plant part is immune from attack, and their ability to survive and multiply rapidly in the harshest of conditions.
- ▼ Contact insecticides penetrate the exoskeleton of insects, and stomach poisons are swallowed by the insect.
- ▼ Organochlorines are persistent, remaining in a stable and active form for periods exceeding a year, and residual, accumulating in the fatty tissues without being excreted or broken down.
- ▼ Organochlorines destabilise the nerve membranes and tend to inhibit the respiratory enzyme cytochrome oxidase.
- ▼ Organophosphates are easily formulated into sprays which coat the surface of leaves, and may penetrate into the epidermal tissues giving a semi-systemic action.
- ▼ The chemical control of insect pests poses four well publicised hazards. Insecticides may be directly poisonous (toxic) to man. Insecticides may accumulate in food chains and have serious, even fatal, effects on top carnivores (including man). Insecticides may reduce populations of beneficial insect species. The use of insecticides encourages the increase of resistance in pest species.
- ▼ Biological control involves the use of other organisms to attack the pest, cultural control involves cultivation techniques which reduce conditions favourable to the pest, and the development of resistant crops involves the breeding-in of resistance to the pest.
- ▼ None of these alone forms a viable strategy for maintaining food levels.
- ▼ They should not be considered as alternatives to each other but as complementary strategies in a balanced and integrated approach to pest management.

Student Activity

Materials

- Microscope and slides of insect mouthparts
e.g. aphid, caterpillar

Procedure

Examine the slides and sketch the differences between sucking and chewing mouthparts

Set Work

Write a paragraph, as to someone who has no understanding of Biology, about why humans should be concerned when birds begin to suffer the effects of persistent pesticides.

Applied Biology

2.7 Biotechnology and the manipulation of reproduction in humans and domestic animals

Reproduction and its hormonal control

Topic Guide

Session: _____

Check List

- ▼ In the sexually mature female mammal regular sexual cycles occur, during which one or more female gametes (ova, singular ovum) develop inside follicles and are released from the ovary, offering the chance of fertilisation if mating occurs
- ▼ These cycles really consist of two closely linked cycles which are synchronised by hormonal interactions.
- ▼ The follicular or ovarian cycle which is concerned with the development and release of ova.
- ▼ The uterine cycle which is concerned with the development of the endometrium (uterus lining) to ensure the highest chance of implantation of a fertilised ovum or zygote.
- ▼ The sexual cycle relies on interactions between four main hormones: the ovarian hormones oestrogen and progesterone, and the pituitary hormones Follicle Stimulating Hormone (FSH), and Luteinising hormone (LH). Although timings of cycle vary between species the same basic pattern holds true.
- ▼ FSH is released from the anterior pituitary and stimulates the development of several follicles (primary oocytes) in the ovary. One or more of these will eventually mature into a Graafian follicle containing the ovum
- ▼ FSH stimulates the production of oestrogen from the follicles within the ovaries. This Oestrogen in turn stimulates the proliferation of the endometrium. At this point oestrogen inhibits FSH (negative feedback control).
- ▼ Oestrogen levels increase until the mid point of the cycle when they bring about a peak of LH production from the anterior pituitary causing ovulation (the release of the ova from Graafian Follicles).
- ▼ LH causes the Graafian Follicle to develop into a corpus luteum which begins to secrete the progesterone and oestrogen.
- ▼ Progesterone stimulates further development of the endometrium to prepare the uterus in case the newly released ovum is fertilised.
- ▼ The other function of progesterone is to inhibit the production of both FSH and LH and hence the development of further follicles.
- ▼ If fertilisation does not occur the corpus luteum degenerates and secretion of progesterone and oestrogen falls. This fall causes the uterine blood vessels to rupture and the uterus lining to degenerate. Decline in progesterone levels causes the inhibition of FSH and LH to be removed and a new cycle can begin.
- ▼ If fertilisation does occur then the corpus luteum does not degenerate but continues to secrete oestrogen and progesterone, maintaining the uterus lining and preventing further follicles from developing. Eventually the developing placenta takes over this endocrine role.
- ▼ Oestrus is a change in the behaviour or appearance of a female mammal which occurs around the time of ovulation, indicating sexual receptiveness to males.
- ▼ Cows attempting to mount other females and then standing still and allowing themselves to be mounted by other females is the best sign of oestrus.
- ▼ Detection of oestrus by humans, allows intervention in the process of reproduction, e.g. by artificial insemination (AI).

Student Activity

Materials

- Microscopes and slides of TS mammalian ovary
- OHT ovarian cycle in human female

Procedure

Make a series of sketches illustrating the different stages of follicle development in the ovarian cycle.

Set Work

List the advantages of artificial insemination over 'natural' fertilisation.

Manipulation and control of reproduction

Session:

▼ Both FSH and LH are commonly used to encourage multiple ovulation for in vitro ('test tube') fertilisation.

Notes:

Applied Biology

2.7 Biotechnology and the manipulation of reproduction in humans and domestic animals

Reproduction and its hormonal control

Topic Guide

Session: _____

Check List

- ▼ Cows are treated with hormone injections to stimulate the development and release of much larger numbers of ova than would naturally occur. As in humans, the main strategy involves using FSH and LH at appropriate times. In other cases a gonadotrophin hormone is used.
- ▼ When ovulation occurs (detected by the signs of oestrus mentioned before, or by calculation of time since LH injection) the cow is artificially inseminated.
- ▼ The fertilised ova are allowed to develop for up to 7 days and are then washed out of the uterus before implantation occurs.
- ▼ The embryos are frozen and stored until required.
- ▼ Embryos are introduced into the uterus of cows other than that from which they were obtained, where they implant and develop until full term. This process is known as 'embryo transfer'.
- ▼ By this process a single high quality cow may produce a very large number of offspring allowing the quality of a herd to improve very quickly.
- ▼ The synchronisation of oestrus cycles in flocks of sheep (ewes) or herds of cows ensures that all animals in the herd come into oestrus on approximately the same day and can be mated or artificially inseminated at the same time.
- ▼ Synchronised oestrus is particularly desirable in ewes, as opposed to cows or sows for two reasons: firstly these animals have distinct breeding seasons and cannot become pregnant outside of this season and secondly, many flocks of sheep live in isolated hill regions. It is much more efficient to herd the sheep together to start treatment and then again for mating or A.I.
- ▼ All ewes in a flock are treated with progesterone for 5-9 days. The hormone is administered in one of three ways: orally, via an implant under the skin, or via a sponge in the vagina.
- ▼ The progesterone is stopped in all ewes on the same day. This removes the inhibition of FSH and LH and allows follicles to start development in the ovaries of the ewes.
- ▼ Several days later the ewes come into oestrus.
- ▼ A ram is obtained and used to fertilise all the sheep at the same time. Alternatively A.I. may be used. This may ensure higher fertilisation rates, be quicker, prevent the spread of disease and allow semen to be chosen from a wide range of rams.
- ▼ All ewes who were fertilised produce lambs at approximately the same time.
- ▼ Even though a high yielding cow may produce over 40 litres of milk per day (up to 12 000 litres/year) there is still an interest in further boosting production.
- ▼ The hormone BST, (bovine somatotrophin), is a pituitary growth hormone which occurs naturally in young cattle. Its function is to increase cell division, protein synthesis and growth. Trials with BST, which is now produced by genetically engineered bacteria, showed that injecting dairy cows with the hormone led to a 10-15% increase in milk yield, presumably through an increase in growth and activity of milk producing tissue in the udder.

Student Activity

Procedure

Debate the moral and ethical issues associated with using biotechnology to manipulate reproduction in animals.

Set Work

List the moral and ethical issues associated with using biotechnology to manipulate reproduction in animals.

Topic Guide



- 3.1 Bacteria and viruses as pathogenic microorganisms**
- 3.2 Parasites show structural and physiological adaptations to infect and survive inside new hosts**
- 3.3 Mammalian blood in defence against disease**
- 3.4 The transfer of genetic information during cell division**
- 3.5 Genes and the genetic code**
- 3.6 Gene technology may be used in combating disease**
- 3.7 Non communicable disease including heart disease and cancer**
- 3.8 Disease diagnosis**
- 3.9 Drugs in the control and treatment of disease**

Pathogens and Disease

3.1 Bacteria and viruses as pathogenic microorganisms

Bacteria
Viruses

Topic Guide

Session: _____

Check List

- ▼ Bacteria reproduce asexually by binary fission (one cell dividing by mitosis to form two identical daughter cells). The increase in numbers of a bacterial population is referred to as bacterial growth.
- ▼ The nature of this growth process is such that a characteristic sigmoid growth curve can be plotted with numbers of bacteria against time.
- ▼ The curve has four main phases:
 - ▼ Lag phase in which the bacterial culture is getting started, but replication is not occurring at a maximum rate.
 - ▼ Log phase which is the period of maximum growth. The population increases by doubling at intervals (e.g. 2->4->8->16->32->64 etc.).
 - ▼ Stationary phase. After a brief period of growth at a reduced rate, bacterial growth ceases and the numbers stay constant.
 - ▼ Decline phase. In this final part of the curve bacteria stop dividing and the death rate increases. Numbers may eventually fall to zero.
- ▼ In natural habitats such curves are unlikely to be found as ideal conditions for exponential growth and a habitat free of competitors or predators is rarely found.
- ▼ Viruses are structures from 20-300nm in size, which contain genetic material in the form of DNA or RNA (never both) and infect cells of other organisms (prokaryotic and eukaryotic).
- ▼ Viruses are incapable of reproducing themselves outside the environment of a host cell.
- ▼ All viruses have similar structural features which include a central core of genetic material (DNA or RNA) and a capsid or protective protein coat around the core formed of units called capsomeres.
- ▼ HIV is a virus which causes the disease known as AIDS (Acquired Immune Deficiency Syndrome).
- ▼ HIV infects a type of white blood cell known as the T-helper lymphocyte or CD4 cell. In doing so it severely damages the immune system of infected people, making them highly susceptible to infection and death from a range of infectious diseases and cancers.
- ▼ HIV belongs to a group of viruses known as retroviruses. The main feature of such viruses is the presence of RNA rather than DNA as the genetic material in the core of the virus. The RNA is encapsulated in layers of proteins, glycoproteins and lipids.
- ▼ Like all viruses, HIV is incapable of independent replication. It relies on inserting its genetic material into the DNA of human T-helper cells and allowing these cells to manufacture new copies of the viral RNA and proteins necessary for assembling new viruses.

Student Activity

Materials

- ☐ OHT Bacterium
- ☐ OHT HIV virus

Procedure

Examine how the sigmoid growth curve of bacteria would be altered with changing conditions.

Set Work

Write a paragraph on viruses as 'rogue genes'

Notes:

Pathogens and Disease

3.1 Bacteria and viruses as pathogenic microorganisms

The association of microorganisms with disease

Topic Guide

Session: _____

Check List

- ▼ Koch established three postulates (criteria) which must be fulfilled if an infective cause for a disease (such as a bacteria or virus) is to be proven.
- ▼ The organism must be sufficiently abundant in every case to account for the disease
- ▼ The organism associated with the disease can be cultured (grown in the laboratory) artificially in pure culture
- ▼ The cultivated organism produces the disease upon inoculation into another member of the same species.
- ▼ Disease causing microorganisms (pathogens) can enter the body through the skin when it is cut or damaged. HIV is most commonly transmitted during sexual intercourse when infected semen from one partner enters the blood of another via a cut or tear in the epithelium of the vagina or rectum.
- ▼ Through the mucous membranes of the respiratory tract when air containing droplets of infectious material are breathed in. This is the way in which *Mycobacterium tuberculosis* the bacterium which causes TB, and cold and influenza viruses, are transmitted.
- ▼ Through the digestive tract when food or water containing microorganisms is taken in. *Salmonella enteritidis*, responsible for food poisoning enters when undercooked contaminated food is eaten.
- ▼ Microorganisms cause disease in a variety of different ways. In some cases the microorganisms actually damage and destroy the host cells. In other cases the microorganisms themselves do not directly cause harm, but produce toxins which are harmful. In still others it is the body's own immune response to the presence of the micro-organism which produces the symptoms.
- ▼ HIV infection is an example of a pathogen causing direct damage to human cells which in turn affect critical functions within the body. As discussed above, HIV only infects one type of cell: the T-helper lymphocyte, cells which play a vital role in the human immune system.
- ▼ As the infection progresses more and more T-helper lymphocytes are infected and destroyed resulting in the condition of Acquired Immune Deficiency Syndrome (AIDS). Here the patient is unable to mount immune responses against common pathogens since the T-helper lymphocytes 'help' B and T lymphocytes to work.
- ▼ *Salmonella* bacteria also cause direct damage to cells. When these bacteria arrive in the intestine they are taken up by epithelial cells. These invaded cells then detach from the intestine wall, creating inflamed lesions. The effect of this is to cause the secretion of large amounts of watery fluid into the lumen of the gut, resulting in diarrhoea
- ▼ Toxins produced by bacteria may be significant in causing disease e.g. cholera
- ▼ *Mycobacterium tuberculosis* falls into the third basic category described above: that is the effect of the bacterium on the lungs (and indeed other parts of the body) is a result of an immune response by the body to the bacterium.

Student Activity

Procedure

Construct a diagram reviewing the methods of entry, and methods of causing disease by microorganisms.

Set Work

List some difficulties in applying Koch's postulates.

Notes: _____

Pathogens and Disease

3.2 Parasites show structural and physiological adaptations to infect new hosts

Parasites and parasitism

Topic Guide

Session: _____

Check List

- ▼ A parasite is an organism which lives on or in a host, causing harm to the host whilst gaining nutritional and other benefits from it.
- ▼ Parasitic organisms often show a range of unique structural and functional adaptations to their lifestyle which distinguish them from non-parasitic relatives. Some key features include:
- ▼ Specialised reproductive strategies aimed at overcoming the unpredictability of finding new hosts including producing vast quantities of offspring during phases of asexual reproduction.
- ▼ Modification of mouthparts, digestive systems and enzymes to allow attachment to the host and utilisation of host's food supply, blood or tissues.
- ▼ Features to resist attack by host enzymes or immune system. This may include having a tough protective cuticle to resist attack by host enzymes as well as strategies to evade the immune system.
- ▼ Reduction of unnecessary sensory organs and locomotory organs in the adult stages (especially in larger parasites).
- ▼ *Plasmodium* is a single celled intracellular parasite of the Protoctist kingdom (Class Protozoa) which causes the disease malaria in humans.
- ▼ It has a complex life cycle which involves two hosts: the human and the female mosquito (*Anopheles sp.*), both of which are essential for its development. Inside the human *Plasmodium* feeds on haemoglobin inside the red blood cells.
- ▼ The life cycle of *Plasmodium* includes an asexual reproductive phase in the human (in liver cells and then red blood cells) and a sexual reproductive phase in the female mosquito.
- ▼ The asexual phase produces enormous quantities of parasites (merozoite stage), and the sexual phase generates the genetic diversity which is essential for evading the hosts immune system.
- ▼ When the mosquito takes a blood meal from a human. Malaria parasites in the sporozoite form are injected into the human as the female mosquito in the anti-coagulant saliva from the salivary glands, whilst *Plasmodium* gametes are taken up in the blood on which the mosquito feeds.
- ▼ *Schistosoma* is the name of a genus of parasitic flatworms (Platyhelminthes: Class Trematoda) which cause the disease schistosomiasis (formerly known as Bilharzia) in humans.
- ▼ Like *Plasmodium*, *Schistosoma* has a complex life cycle involving two hosts. In this case certain fresh water snails are the non-human hosts.
- ▼ The complexity of the life cycle and relatively small chance of finding a new host is overcome by the enormous numbers of larvae produced during asexual reproduction.

Student Activity

Materials

- Microscopes and slides of *Plasmodium* and *Schistosoma*

- OHT Life cycle of *Plasmodium*
- OHT Life cycle of *Schistosoma*

Procedure

Summarise the similarities between the two life cycles.

Set Work

Explain in what way locomotory and other structures are 'reduced' in parasites i.e do they possess them and lose them, or is there another explanation.

Notes: _____

Pathogens and Disease

3.3 Mammalian blood in defence against disease

General mechanism of defence against disease

Topic Guide

Session: _____

Check List

- ▼ If the skin is broken the blood clotting process is rapidly activated to plug the wound and prevent pathogen entry.
- ▼ The mucous membranes of nose and respiratory tract trap pathogens in the sticky mucus, which is moved away from the delicate lining of the alveoli by cilia.
- ▼ Anti-bacterial enzymes are found in saliva, sweat and tears
- ▼ Stomach acid (pH 2) kills pathogens in the food.
- ▼ Non-specific immunity is targeted at foreign material in general. Involves phagocytosis and inflammation and is immediate.
- ▼ Phagocytosis is the process by which pathogens, other foreign material and dead or infected cells are engulfed and digested by phagocytes. These phagocytes are non-specific in their actions.
- ▼ Specific immunity uses B and T lymphocytes to target specific pathogens. Such responses are more complex and are often delayed.
- ▼ Blood clotting involves a series of stages, (a cascade) catalysed by chemicals known as 'clotting factors'.
- ▼ Platelets and damaged tissues at the wound site release a number of substances including thromboplastin, several clotting factors (such as factor VII and factor X) and calcium ions
- ▼ Thromboplastin and other substances catalyse the conversion of prothrombin (inactive) to thrombin, an active protease enzyme. Calcium ions are necessary for this stage.
- ▼ Thrombin converts the soluble protein fibrinogen in the plasma to its insoluble form fibrin by hydrolysis.
- ▼ The insoluble fibrin forms a dense mesh of cross-linked fibres which traps platelets and red blood cells around the wound. Platelets are activated and become sticky, attaching to the fibrin fibres forming a dense plug and also to the walls of any damaged vessels.
- ▼ Gradually the fibrin fibres pull the clot in tighter so that it completely plugs the wound.

Student Activity

Materials

- Microscope and slides of human blood smears

□ OHT Phagocytosis

□ OHT Blood clotting

Procedure

Use □ OHT Phagocytosis and □ OHT Blood clotting to review these topics.

Observe phagocytes in blood smears.

Set Work

Write a paragraph on the ways in which blood is prevented from clotting in undamaged blood vessels.

Notes: _____

Pathogens and Disease

3.3 Mammalian blood in defence against disease

Principles of immunology

Topic Guide

Session: _____

Check List

- ▼ Antigens are defined as molecules, which, when foreign to an individual, stimulate the production of antibodies by B-lymphocytes.
- ▼ Antibodies are protein molecules known as immunoglobulins which are secreted by activated B-lymphocytes when a specific antigen is encountered.
- ▼ There are two main types of immune response: the Humoral Response which is associated with B-lymphocytes and involves the production of antibodies, and the Cellular or cell-mediated response which is associated with the T-lymphocytes.
- ▼ Binding of antigen to antigen receptor activates the B-lymphocyte and causes it to divide by mitosis producing a clone of identical B-lymphocytes.
- ▼ Most of the B-lymphocytes turn into plasma cells and the rest turn into memory cells.
- ▼ The plasma cells begin to secrete specific antibodies against the pathogen concerned.
- ▼ Once the foreign material has been destroyed, the plasma cells eventually die and antibodies stop being secreted. But the memory cells remain in the lymph nodes and the circulation.
- ▼ If a subsequent infection with the same pathogen occurs then some of these will turn into plasma cells and start producing antibodies. This is the basis of natural active immunity to diseases and underlies the practice of immunisation or vaccination which are methods of artificial active immunity.
- ▼ T-lymphocytes do not produce antibodies but work directly on infected cells.
- ▼ Antigens of pathogen which are presented on a body cell come into contact with specific T-lymphocyte receptors which recognise them.
- ▼ T-lymphocyte binds to presented antigen on infected cell via antigen receptor.
- ▼ T-lymphocyte is activated and divides to form a clone of identical T-lymphocytes which enter the circulation and can attach to other infected cells.
- ▼ T-lymphocytes destroy the pathogens inside the cells to which they are bound. Some do this by releasing lytic enzymes into the infected human cells. Others release chemical messengers called cytokines which attract phagocytes to engulf the cells.
- ▼ Some T-lymphocytes then turn into memory cells which remain in circulation and in the lymph nodes in case of further infection.

Student Activity

Materials

- OHT B-lymphocytes and T-lymphocytes

Procedure

Revise the complex chain of events using □ OHT B-lymphocytes and T-lymphocytes. The link with 12.1 Bacteria and Viruses and HIV should provide a sharp focus for students.

Set Work

List reasons why people can vary so much in their susceptibility to infectious diseases

Notes: _____

Pathogens and Disease

3.3 Mammalian blood in defence against disease

Passive and active immunity

Topic Guide

Session: _____

Check List

- ▼ Passive immunity occurs when the individual plays no role in actually producing the antibodies against that disease. Since no memory cells are formed, such passive immunity will only last a few weeks or months, after which time the antibodies will be broken down by the body and stop working.
- ▼ Natural passive immunity occurs when antibodies are passed from mother to fetus via the placenta or from mother to baby via the colostrum (first milk). These antibodies protect the baby from diseases which it may encounter in the first few months of life before its own immune system starts functioning properly.
- ▼ Artificial passive immunity occurs when antibodies produced in another organism are injected into a person to give them short term protection against a specific disease.
- ▼ Antibodies required for passive immunisation can be produced as monoclonal antibodies in the laboratory.
- ▼ The immunity to a disease which follows a natural infection with a pathogen is known as natural active immunity.
- ▼ Immunisation includes vaccination (which produces artificial active immunity, often for life) and the provision of antibodies (artificial passive immunity) which provides immunity for a short period of time only.
- ▼ Vaccination is a process by which immunity to a disease is achieved without experiencing a natural infection with that disease. A very small dose of antigens of the pathogen to which immunity is required is given via an injection or an oral vaccine.
- ▼ Vaccines containing a 'toxin' produced by the pathogen but not the whole pathogen. e.g. diphtheria.
- ▼ Vaccines containing dead (heat killed) pathogens. e.g. influenza. Even though the organism is dead its antigens can still be recognised and an immune response made against them.
- ▼ Vaccines containing a modified or weakened (attenuated) form of the pathogen. e.g. polio, *Rubella*, TB.
- ▼ Genetically engineered vaccines may contain live pathogens which have been genetically modified or attenuated by removing or 'switching off' potentially harmful genes. Or the gene or genes coding for the antigenic proteins on the surface of a pathogen may be removed from the pathogen, and inserted into another form of bacterium or yeast. This can allow for the microbial production of vast amounts of antigen for use in vaccines.

Student Activity

Procedure

Review the effectiveness of vaccination in the prevention of infectious diseases, relating it to the proportion of the group who could have died as a result of infectious diseases if they had not been vaccinated.

Set Work

List the disadvantages of vaccination programmes.

Notes:

Pathogens and Disease

3.4 The transfer of genetic information from cell to cell during division

Mitosis

The cell cycle

Topic Guide

Session: _____

Check List

- ▼ Mitosis is nuclear division in which the daughter nuclei have the same chromosome number as the parent nucleus, typically diploid nuclei producing diploid daughter nuclei.
- ▼ The daughter nuclei are therefore genetically identical to the nucleus of the cell that produced them (except for any mutations that may occur).
- ▼ Mitotic cell division is seen in growth, repair and asexual reproduction.
- ▼ As a result of mitosis in the growth of an organism from the fertilised egg all cells therefore are genetically identical (totipotent).
- ▼ Similarly, offspring of asexual reproduction lack genetic variation.
- ▼ The production of genetically identical cells retains the full set of genetic information needed for the replacement of any cells in repair processes, and in the asexual reproduction of organisms it allows for the rapid exploitation of optimum conditions where any variation would be disadvantageous.
- ▼ The DNA replicates in interphase so that in the first stage of mitosis (prophase) the chromosomes appear as double structures of two chromatids (daughter chromosomes) joined at the centromere.
- ▼ The nuclear spindle apparatus(NSA) forms from the dissolution of the nuclear membrane and the centrioles replicate and migrate to either pole of the NSA.
- ▼ The centromeres align on the equator of the NSA (metaphase).
- ▼ The centromeres divide and the chromatids are moved to the opposite poles (anaphase).
- ▼ Two new daughter nuclei are formed (telophase).
- ▼ The cytoplasm is divided between two daughter cells by cleavage in animal cells and the laying down of new cell walls in bacteria and plants
- ▼ The formation of two new cells completes the cell cycle.

Student Activity

Materials

- Microscopes
- Slides of root tip squashes
- Practical Work Sheet:
Preparation of an onion root tip squash to show stages in mitosis
- OHT Root tip squash
- OHT Normal Human Karyotype

Procedure

Observe slides of root tip squashes.

Use □ OHT Root tip squash for guidance.

Make drawings of prophase, metaphase, anaphase and telophase nuclei.

Counting nuclei at the same stage of mitosis gives an estimation of the duration of that stage, as the longer the stage the more nuclei are likely to be 'caught' in it at the time the cells are killed.

Look for similarities between the root tip cell nucleus and the human nucleus.

Set work

Determine at what stage in mitosis the photograph of the human nucleus and chromosomes was taken, and from what type of cell. Explain your reasons.

Notes:

Meiosis

Session:

- ▼ Each gamete of a sexually reproducing organism contains one set of chromosomes (haploid).
- ▼ When two such gametes fuse in fertilisation they produce a zygote with two sets of chromosomes (diploid).
- ▼ Chromosomes in a diploid nucleus thus occur in pairs, each member of a pair carrying the same genes in the same relative positions (homologous chromosomes).
- ▼ Slight variations (alleles) of a gene at a particular locus can arise by mutation, so that an organism can either have an identical pair of genes at a locus

- ▼ Pairs of sex determining chromosomes are only partially homologous e.g. the human Y chromosome has a short non-homologous arm which carries the male determining genes.
- ▼ The production of haploid gametes from a diploid cell requires a reduction division (meiosis) in which the chromosome number is halved from diploid to haploid.
- ▼ The production of haploid gametes ensures the maintenance of the diploid chromosome number in subsequent generations.

Materials

- ## Procedure

Examine the pairs of homologous chromosomes of the Human Karyotype. Note that before the development of staining techniques that revealed differences in dark staining bands, it was not possible to pair up the chromosomes into homologous pairs with any confidence.

Set work

Explain why chromosome studies are carried out on squashes of cells and not sections.

Notes:

Replication of DNA

Session:

- ▼ The nucleus of cells contains mostly DNA and protein.
- ▼ The DNA content doubles in cells just prior to dividing.
- ▼ It was also found that, in the formation of gametes (sperms and eggs), the amount of DNA halved. This evidence underline its central role in inheritance.
- ▼ Now it has become possible to analyse the molecular structure of DNA, to unravel the chemical code it contains, and to transfer pieces of this code between one organism and another in the process known as genetic engineering.
- ▼ DNA and RNA are long chain polynucleotide molecules with repeating sub-units of nucleotides.
- ▼ Nucleotides consist of a 5 carbon sugar, a phosphate and an organic base.
- ▼ In DNA the sugar is deoxyribose and the organic base can be one of adenine, thymine, cytosine and guanine.
- ▼ In RNA the sugar is ribose and the organic base can be one of adenine, uracil, cytosine and guanine.
- ▼ The DNA molecule is a double stranded helix with the two strands held together by hydrogen bonds between complementary base pairs adenine-thymine and cytosine-guanine.
- ▼ RNA is a single stranded helix with base pairs of adenine-uracil and cytosine-guanine.
- ▼ There are three types of RNA - messenger, transfer and ribosomal.
- ▼ DNA carries out semi-conservative replication in which each strand of the original molecule acts as a template for the construction of a new one, so that each new double stranded molecule of DNA consists of one original strand and a new complementary strand.

Write a paragraph to explain what is meant by semi-conservative replication of DNA.

Notes:

Pathogens and Disease

3.5 Genes and the genetic code

Protein synthesis

Topic Guide

Session: _____

Check List

- ▼ The sequence of bases (and hence nucleotides) on one strand of the DNA molecule carries the genetic code for the synthesis of proteins and hence ultimately living cells.
- ▼ Three bases (a triplet codon) code for one amino acid.
- ▼ There are 64 possible combinations of any three from four bases, but only 20 amino acids to be coded for.
- ▼ Amino acids are coded for by more than one triplet codon and the code is said to be 'redundant' or 'degenerate'.
- ▼ A gene is a sequence of nucleotides of a DNA molecule which codes for a polypeptide.
- ▼ A gene for a polypeptide has a specific 'start' codon which thus sets the order of all the subsequent triplet codons
- ▼ Thus the code is non-overlapping i.e. a base can only be 'used' in one triplet codon.
- ▼ Only a fraction of the nuclear DNA contains genes coding for proteins, the rest consists of so-called 'junk' DNA the function of which is not understood
- ▼ Nuclear DNA with the genetic code never leaves the nucleus in eukaryotic cells.
- ▼ The genetic code for a polypeptide (a gene) is copied (transcribed) into mRNA which leaves the nucleus and associates with the ribosomes.
- ▼ The ribosomes 'read' the genetic message on the mRNA and tRNA deliver amino acids in the sequence determined by the code.
- ▼ There are 20 different types of tRNA, each specific to a particular amino acid.
- ▼ Each tRNA has a specific binding site at which the specific amino acid combines and an anti-codon which matches the specific codon for that particular amino acid.
- ▼ The amino acids combine by means of peptide bonds to give a polypeptide chain.
- ▼ The polypeptides subsequently form proteins.
- ▼ As enzymes are proteins their synthesis is controlled by DNA.

Student Activity

Materials

- ☐ OHTs showing base sequence / gene code
- ☐ OHT Transcription and translation
- ☐ OHT Table of amino acid codons

Procedure

Use OHTs to revise and discuss the genetic code
Can be used to revise transcription and translation.

Set work

Draw out a random sequence of bases of a strand of DNA and annotate it to demonstrate the non-overlapping nature of the genetic code.

Draw out the complementary strand of DNA to the one that you have drawn and annotate to illustrate how only one strand can carry the genetic information.

DNA replication, transcription, and mRNA translation all require enzymes to catalyse their reactions. Explain the particular problem that this represents in terms of protein synthesis.

Notes: _____

Protein synthesis

Session:

- ▼ Some genes necessary for metabolic functions are faulty or absent, e.g. Phenylketonuria (PKU) and cystic fibrosis.
- ▼ In the disease phenylketonuria (PKU) a gene mutation in the DNA coding for the enzyme phenylalanine hydroxylase means that this enzyme is not produced in the liver.
- ▼ Without phenylalanine hydroxylase the body cannot convert the amino acid phenylalanine to the amino acid tyrosine.
- ▼ This results in an accumulation of phenylalanine in the body which severely affects many systems. Untreated sufferers may be both mentally and physically retarded.
- ▼ Cystic fibrosis primarily affects the lungs, pancreas and liver, and is characterised by the production of thick, sticky mucus by epithelial cells in these regions. It usually causes death by early adulthood.

- ▼ The disease is caused by a faulty protein known as CFTR (cystic fibrosis transmembrane regulator) which regulates transmembrane chloride ion channels in epithelial cells.
- ▼ A deletion of 3 bases in the DNA code of the CFTR gene means that one amino acid (phenylalanine) is missing from the CFTR protein which is produced.
- ▼ This change in the primary structure of the protein alters its secondary and tertiary structures.
- ▼ CFTR protein fails to regulate opening of chloride ion channels in epithelial cells
- ▼ Chloride ions stay inside cells, as does water. Sodium ions are attracted in.
- ▼ Mucus produced is thick and sticky
- ▼ Mucus adheres to epithelial cells and causes disease symptoms.

Calculate how many carriers of cystic fibrosis may be known to you

Notes:

Session: _____

3.6 Gene technology may be used in combating disease

Check List

- ▼ Isolation of the gene to be manipulated is the first step
- ▼ It is easier to find and isolate the appropriate mRNA which has been transcribed (copied) from the relevant DNA, than it is to try and isolate the particular length of DNA itself.
- ▼ The enzyme reverse transcriptase can then be used to make a copy of the DNA from the mRNA.
- ▼ Plasmids (circular DNA) are isolated from bacteria.
- ▼ Plasmids are cut open by restriction enzymes and the length of DNA is inserted and the plasmid resealed by ligase enzymes.
- ▼ The genetically modified plasmids are then reintroduced into the bacteria.
- ▼ The bacteria multiply and produce the protein coded for by the inserted DNA.
- ▼ To identify those bacteria which have successfully taken up the modified plasmids marker genes are incorporated along with the required gene.
- ▼ Marker genes include those for antibiotic resistance so that the culture can be flooded with antibiotic and only the bacteria with the marker gene will survive.
- ▼ Two early successes with these techniques were the synthesis of human insulin for the treatment of diabetics, and blood clotting factor VIII for the treatment of haemophiliacs.

Notes:

- ❑ OHT Sticky ends
- ❑ OHT Genetic engineering

Lesson Plan

Use the OHTs for a preliminary explanation of the three enzymes (reverse transcriptase, restriction endonuclease and DNA ligase) which are the basic tools of genetic engineering, and the main steps involved.

Discuss the moral and ethical issues associated with recombinant DNA technology.

Define the following terms: 'recombinant DNA', 'genome', 'plasmid', 'bacteriophage', 'vector'. Defence against disease

Pathogens and Disease

3.7 Non-communicable disease including heart disease and cancer

The biological basis of heart disease

Topic Guide

Session: _____

Check List

- ▼ **Atheroma** (pleural atheromata) are fatty deposits of cholesterol and other fatty materials in the walls of large arteries (especially the aorta, carotid arteries and coronary arteries) leading to the condition atherosclerosis.
- ▼ Lipids accumulate beneath the smooth muscle layer, appearing at first as small fatty streaks which later become fibrous and hardened as connective tissue builds up around the site. The region becomes known as a plaque.
- ▼ Atherosclerosis may lead to high blood pressure which is linked to the loss of elasticity in the arteries, and can lead to an expansion of the artery known as an aneurysm, and thromboses or blood clots which may occur in any diseased artery.
- ▼ Once formed the thrombus may completely block a coronary artery this can lead to myocardial infarction, which occurs when parts of the heart muscle are starved of oxygen and glucose.
- ▼ In other cases the clot may detach itself from the artery wall and enter the circulation. Known as an embolus this clot may then block a vessel in another part of the body. Often they lodge in the pulmonary artery, or in vessels of the brain leading to a stroke.
- ▼ Risk factors associated with coronary heart disease include, high blood cholesterol level, cigarette smoking and its associated carbon monoxide (CO) and nicotine, high blood pressure, lack of exercise, and is much more common in middle aged men.

Student Activity

Notes:

Materials

□ OHT Risk factors in heart disease chart

■ Heart model

Procedure

Observe the model of the heart and coronary blood vessels.

Use □ OHT Risk factors in heart disease chart to evaluate the various risk factors in CHD.

Set Work

Write a paragraph describing why the heart has to have its own coronary circulation in its walls.

Pathogens and Disease

3.7 Non-communicable disease including heart disease and cancer

The biological basis of cancer

Topic Guide

Session: _____

Check List

- ▼ Cancer is not a single disease but rather a complex group of several hundred different diseases which share common characteristics. Chief amongst these is the presence of unregulated cell growth leading to the development of tumours.
- ▼ A tumour is an abnormal mass of tissue, the growth of which is uncoordinated and exceeds that of normal tissue.
- ▼ Tumours are divided into benign and malignant.
- ▼ Malignant tumours invade their surrounding tissues and spread to distant sites via the blood stream, the lymphatics or across body cavities to form secondary tumours, known as metastases
- ▼ Benign tumours usually grow at a slower rate, they never invade organs or blood vessels/lymphatics nor do they metastasize.
- ▼ Tumour cells have the following differences to normal cells: wide variation in the shape and size of the cell large nucleus when compared to the size of the cell, which stains darkly.
- ▼ As a general rule benign tumour cells resemble the normal cell type from which they have arisen, while malignant tumour cells do not.
- ▼ Causes of cancer include chemical carcinogens and radiation which may damage DNA and cause mutations in the genes controlling growth.
- ▼ Carcinogens are chemicals that increases the risk of developing a cancer, and include chemicals found in the tar of cigarette smoke, in car exhaust fumes, and in low levels in some common foods and food additives.
- ▼ Ultra violet light, gamma rays and X-rays are also carcinogenic. It damages DNA and malignant transformation is more likely.
- ▼ The uncontrolled growth seen in cancer, results from mutations in the genes that encode for proteins regulating the normal growth of cells and the cell cycle.
- ▼ A mutation in suppressor genes which code for proteins which suppress cell growth in the normal cell may result in a tumour.
- ▼ Mutations in genes which encode for proteins which control programmed cell death may cause cells to continue to divide to form a tumour.
- ▼ Lung cancer accounts for 19% of all cancers and 27% of cancer deaths in Britain and causes an estimated 1.2 million deaths worldwide in 1999.
- ▼ Skin cancer is correlated with radiation damage by UV light in sunlight, the incidence of melanoma in Black populations is only around 0.6 per 100 000 per year, but as high as 33 per 100 000 per year in those of European descent living in Queensland, Australia.

Student Activity

Materials

□ OHT Cancer statistics

Procedure

Use □ OHT Cancer statistics to evaluate the risk factors involved in cancer.

Set Work

Write a paragraph explaining why life long smokers who live to a ripe old age with no ill effects are not significant in the debate about the health risks associated with smoking.

Notes: _____

Enzymes and diagnosis

Session:

▼ Chronic pancreatitis can be diagnosed by measuring the level of pancreatic enzymes in the intestine. In all cases the levels of pancreatic enzymes are lower than in normal patients.

Notes:

Enzymes and diagnosis

Session:

▼ Enzyme analytical reactors use soluble enzymes linked to dyes which test hundreds of samples per day and give results on the basis of colour changes.

Notes:

From your results calculate how much more sensitive the Clinistix test strips are than the Benedict's test for detecting the presence of glucose in a solution.

Topic Guide

Session: _____

Pathogens and Disease

3.9 Drugs in the control and treatment of disease

Betablockers

Antibiotics

Monoclonal antibodies

Check List

- ▼ A drug may be defined as any chemical which has an effect on the way in which the body works, and on any pathogens in the body.
- ▼ Beta blockers (or b-adrenergic antagonists) are a group of drugs used for the treatment of high blood pressure and angina (pain in the chest, coming from the heart).
- ▼ They reduce cardiac output by slowing heart rate and reducing the force of contraction so that less blood is forced through the vessels per minute.
- ▼ An antibiotic is a molecule produced by one microorganism which is capable of destroying or inhibiting the growth of another microorganism. Most commonly they are produced by fungi or bacteria to act on other bacteria.
- ▼ Antibiotics work in several ways but there are two main categories: bacteriostatic antibiotics inhibit the growth and replication of bacteria; bacteriocidal antibiotics kill other bacteria.
- ▼ Antibiotics use one or more of the four main targets listed below to attack bacterial cells:
- ▼ Interfering with formation of the bacterial cell wall.
- ▼ Limiting or preventing protein synthesis, especially the synthesis of enzymes.
- ▼ Limiting or preventing nucleic acid synthesis and hence cell division.
- ▼ Damaging bacterial cell membranes
- ▼ Monoclonal antibodies are antibodies grown in the laboratory from a single B-lymphocyte clone. All the antibodies produced from such a clone are identical and will thus be specific to one antigen.
- ▼ Monoclonal antibodies have many potential uses in the control and treatment of disease, and other applications: e.g. in the pregnancy testing kit where monoclonal antibodies detect the presence of the hormone HCG in the urine of a pregnant woman.
- ▼ Monoclonal antibodies can detect the presence of cancer cells whose surface antigens differ from those of normal human cells.
- ▼ Monoclonal antibodies with fluorescent or radioactive tags could be injected into the body and used to detect cancer cells.
- ▼ Toxic drugs or radioactive isotopes can be attached to monoclonal antibodies specific to cancer cells. These then attach to the target cells destroy them, leaving non-cancerous cells unharmed.
- ▼ The specificity of monoclonal antibodies make them ideal tools in the diagnosis of infectious diseases. Such tests are currently in use for HIV and the Herpes viruses.

Student Activity

Materials

- Correct conditions for use of microbiological techniques.
- OHT effect of antibiotics on growth of bacteria

Procedure

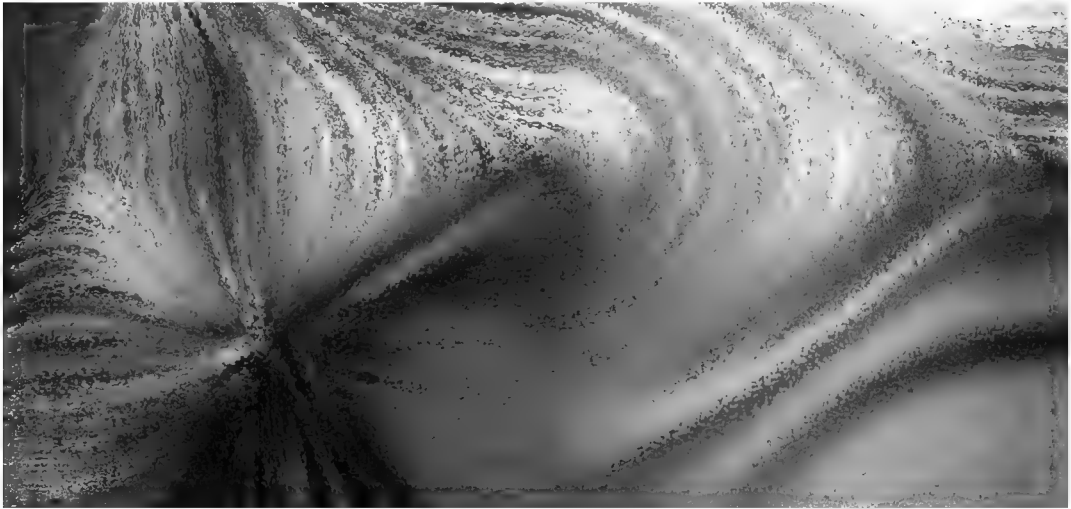
If correct conditions are available for use of microbiological techniques, a plate as illustrated on □ OHT effect of antibiotics on growth of bacteria may be produced.

Set Work

Make a sketch to illustrate the nature of monoclonal antibodies.

Notes: _____

Biology Illustrations



- 1 - Molecules, Cells & Systems**
- 2 - Making Use of Biology**
- 3 - Pathogens & Disease**

FELTHAM PRESS

Illustrations

Contents List

1 Molecules, Cells & Systems

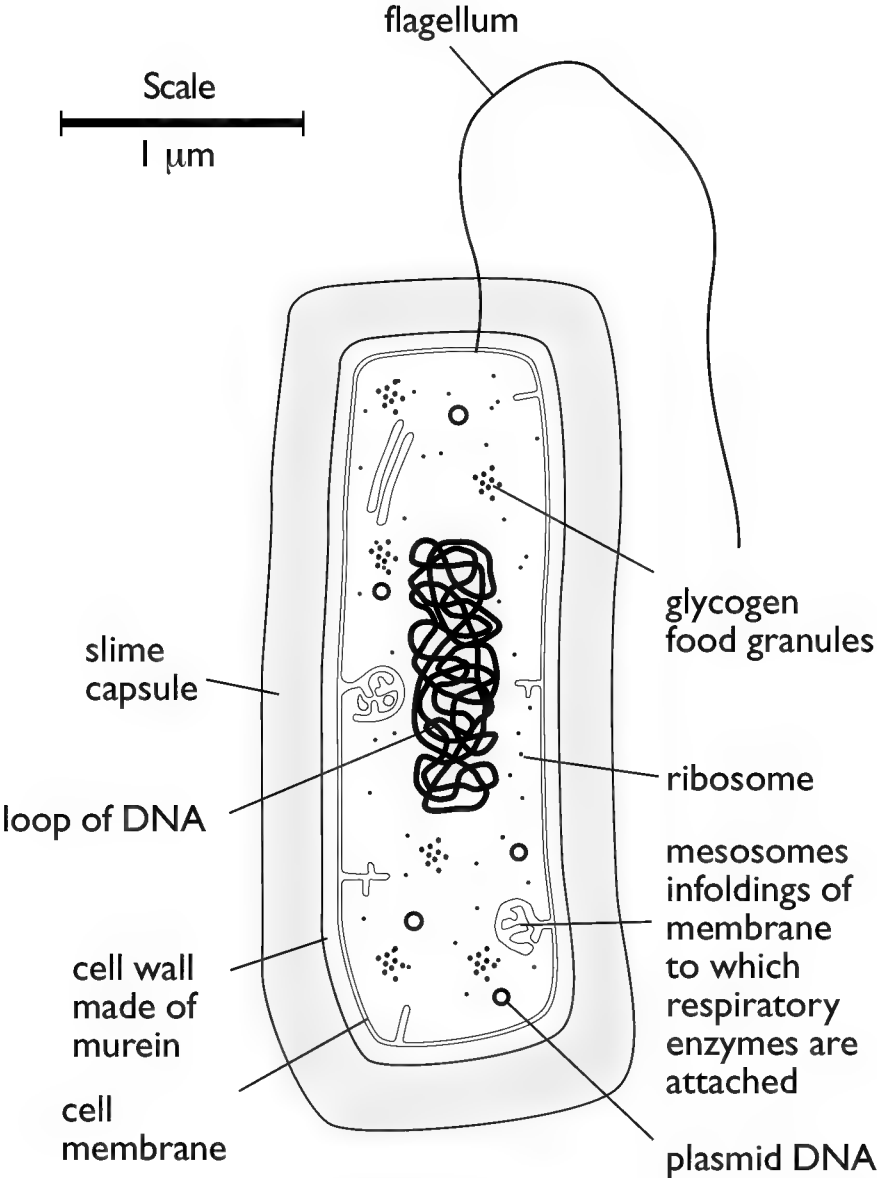
- 1 Prokaryotic cell
- 2 Photomicrograph animal cell
- 3 Photomicrograph plant cell
- 4 The use of graticule and stage micrometer
[Light micrograph (see either 2 or 3)]
- 5 Electron micrograph (human liver cell)
- 6 Magnification and resolution
- 7 Electron micrograph (plant cell)
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2 Making Use of Biology

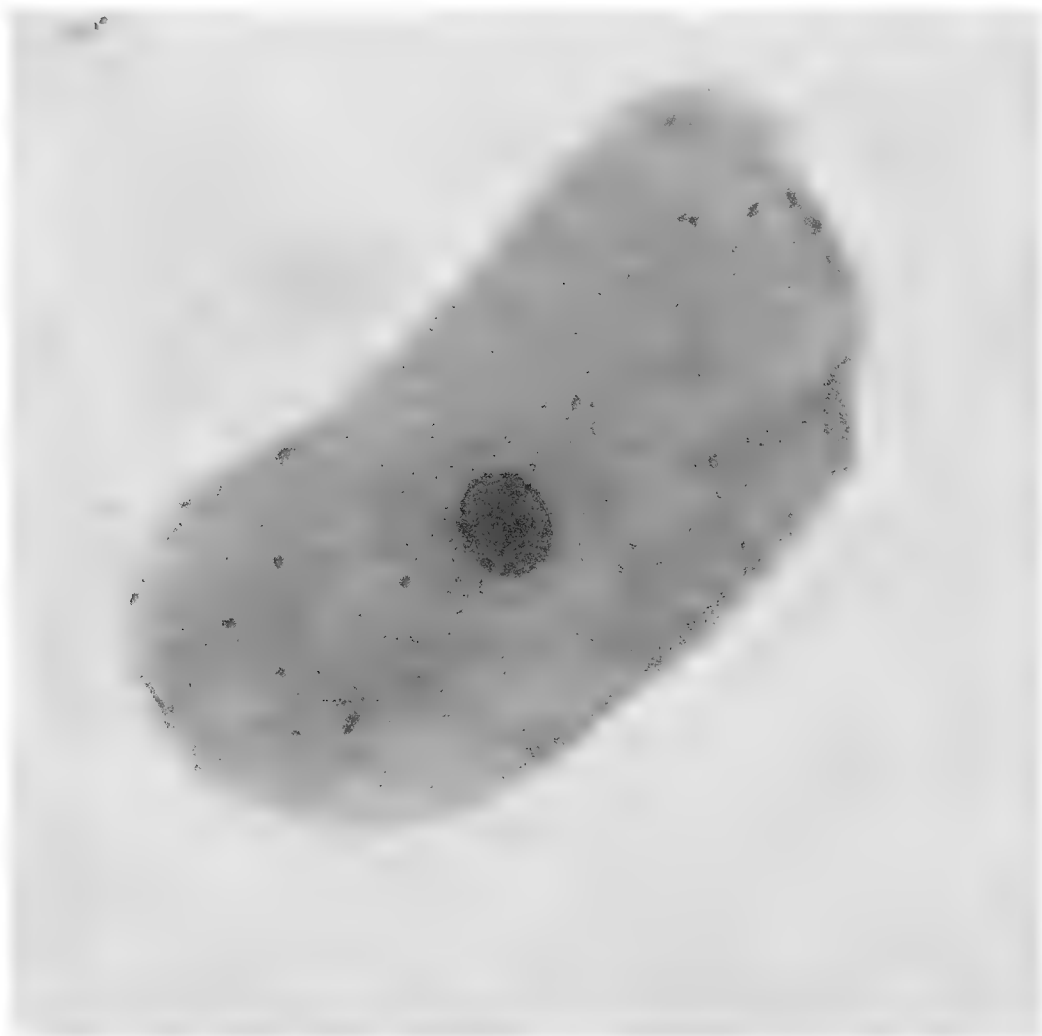
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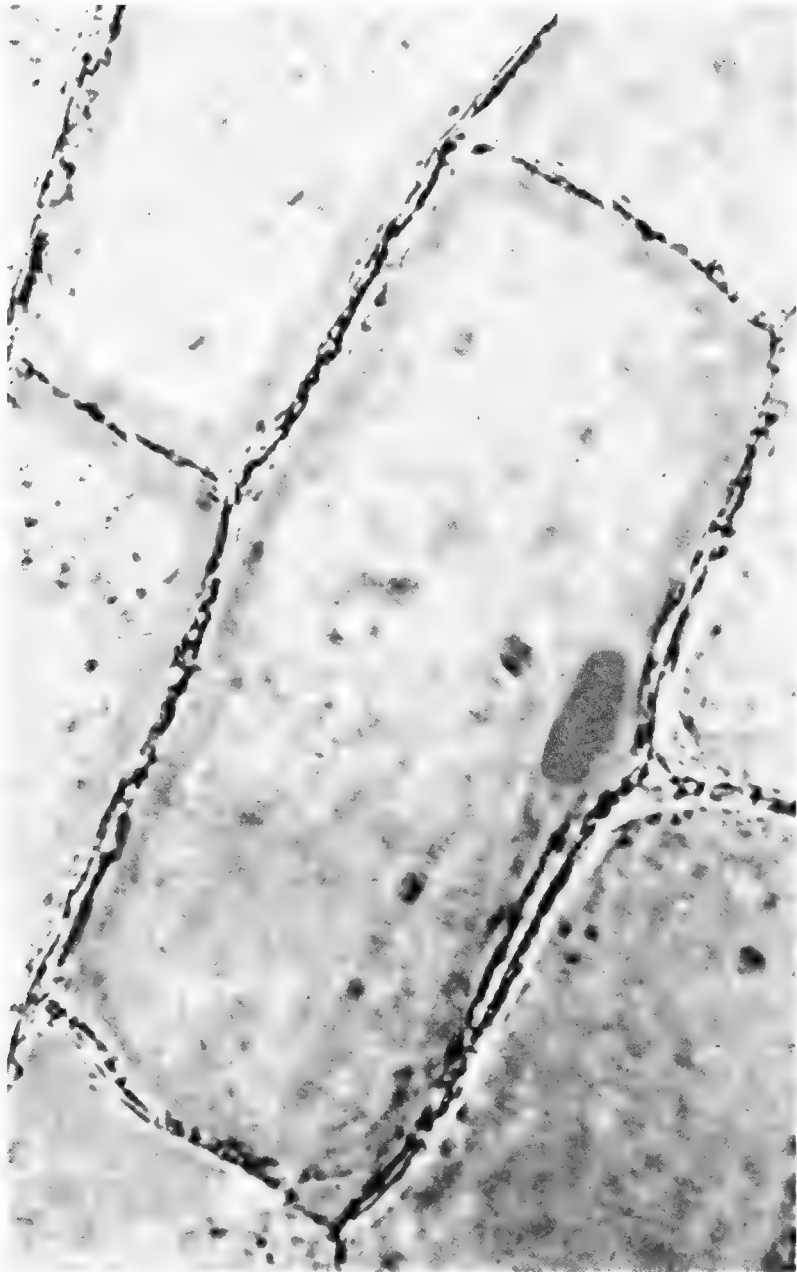
Prokaryotic Cell

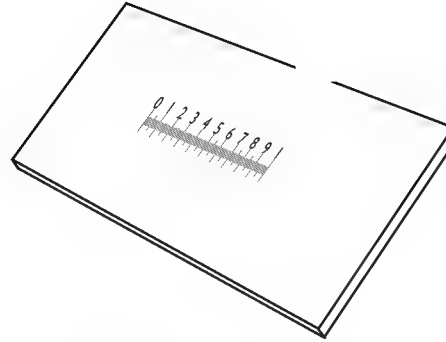
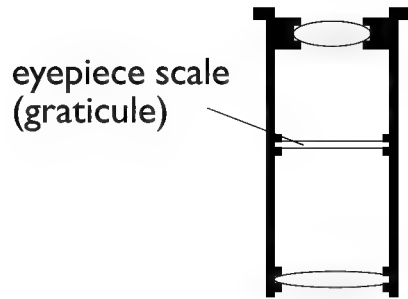


Photomicrograph of human cheek cell

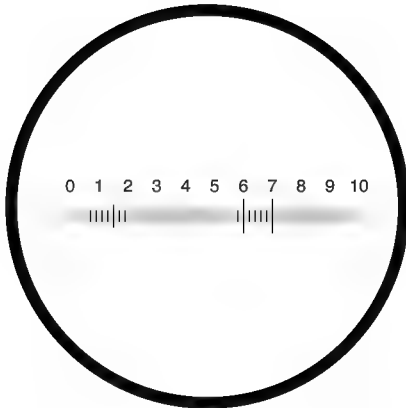


Photomicrograph of plant cells

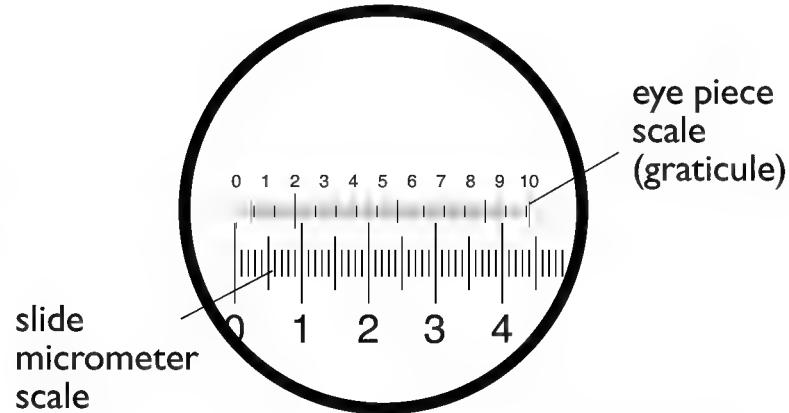




Scale in mm etched upon a microscope slide or a printed acetate sheet (slide micrometer)

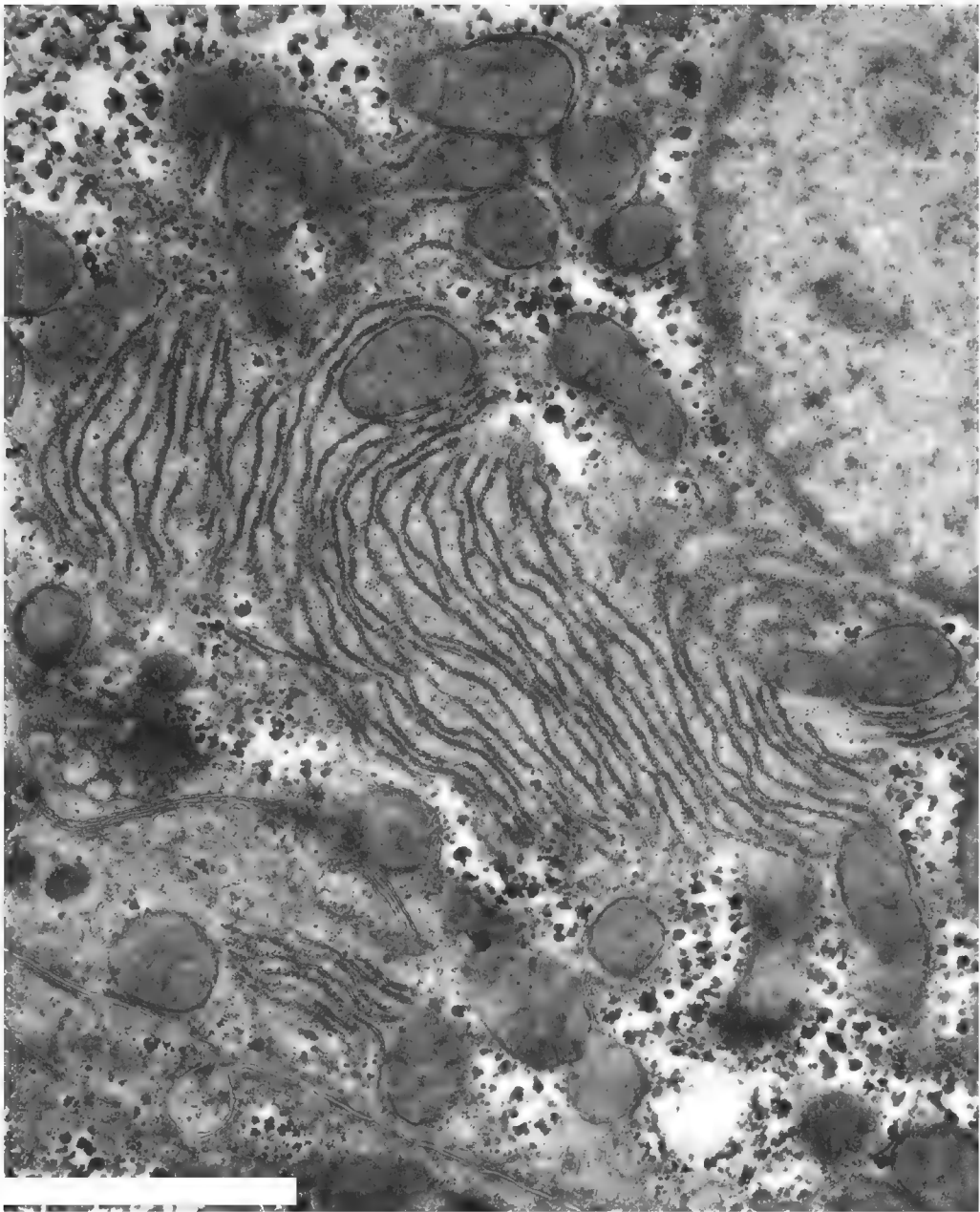


Eyepiece scale as seen when held up to the light away from the microscope.



Accurate measurements can be achieved using the two scales in combination through the lenses of the microscope

Electron micrograph of human liver cell



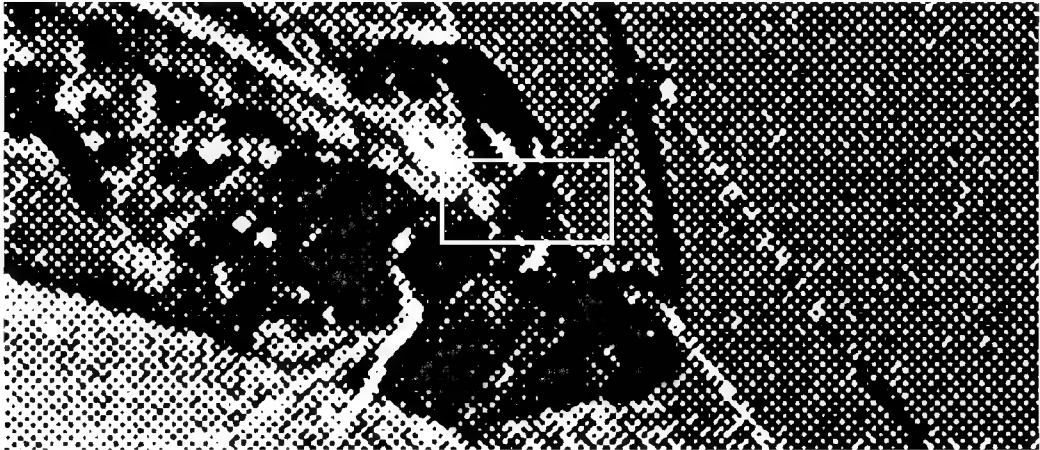
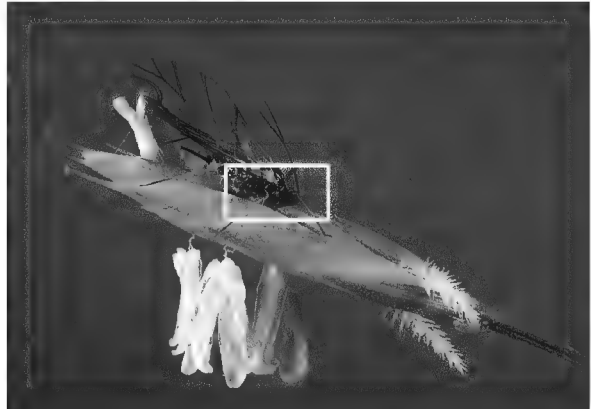
Magnification and Resolution

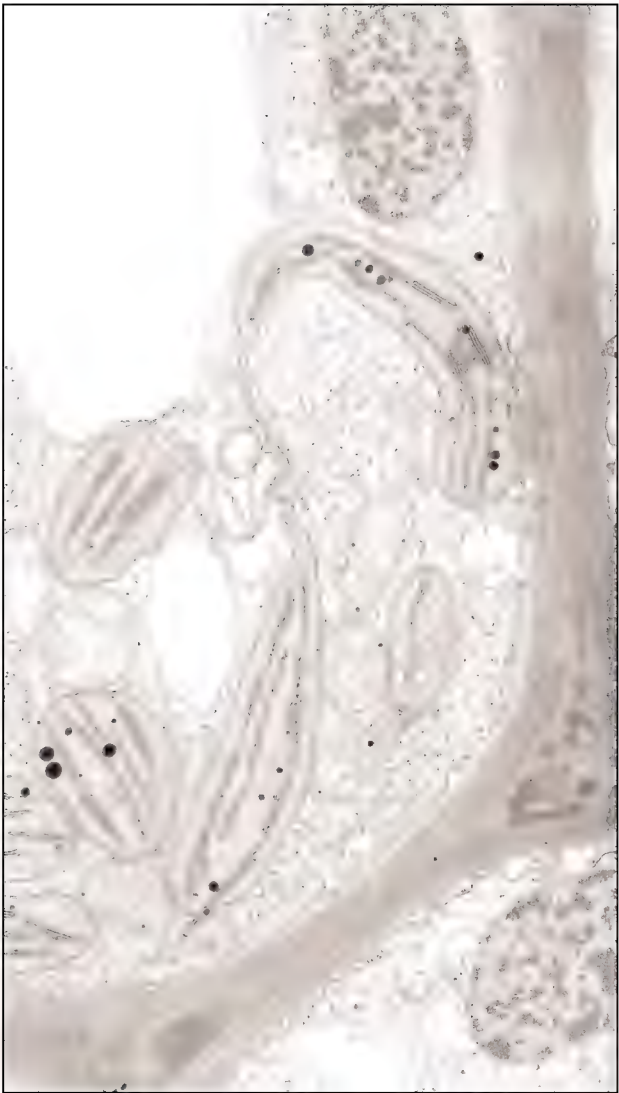
The image is composed of dots.

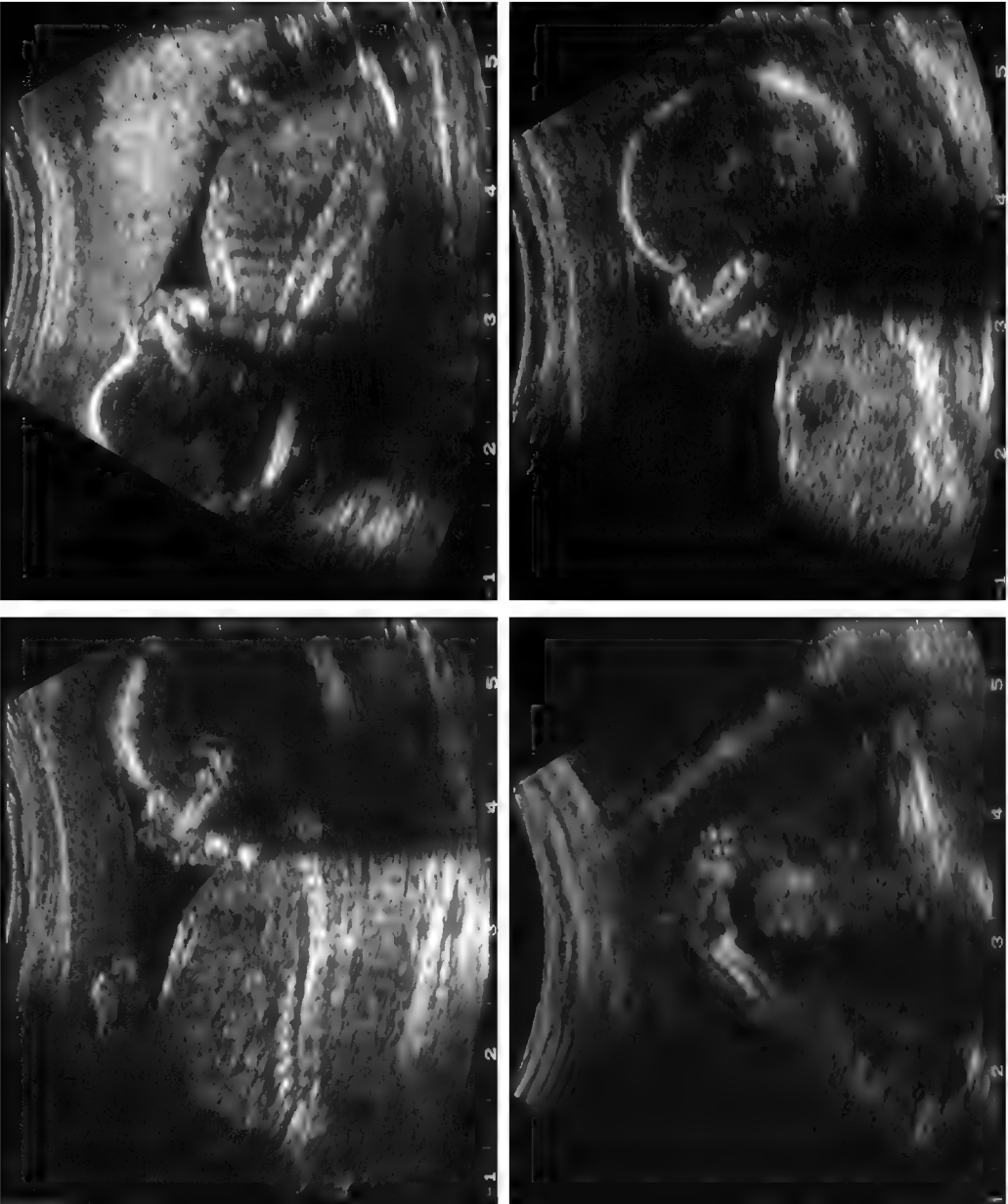
No amount of **magnification** will reveal any more detail or information ie; nothing more can be **resolved**.

Magnification increases the apparent size of an object.

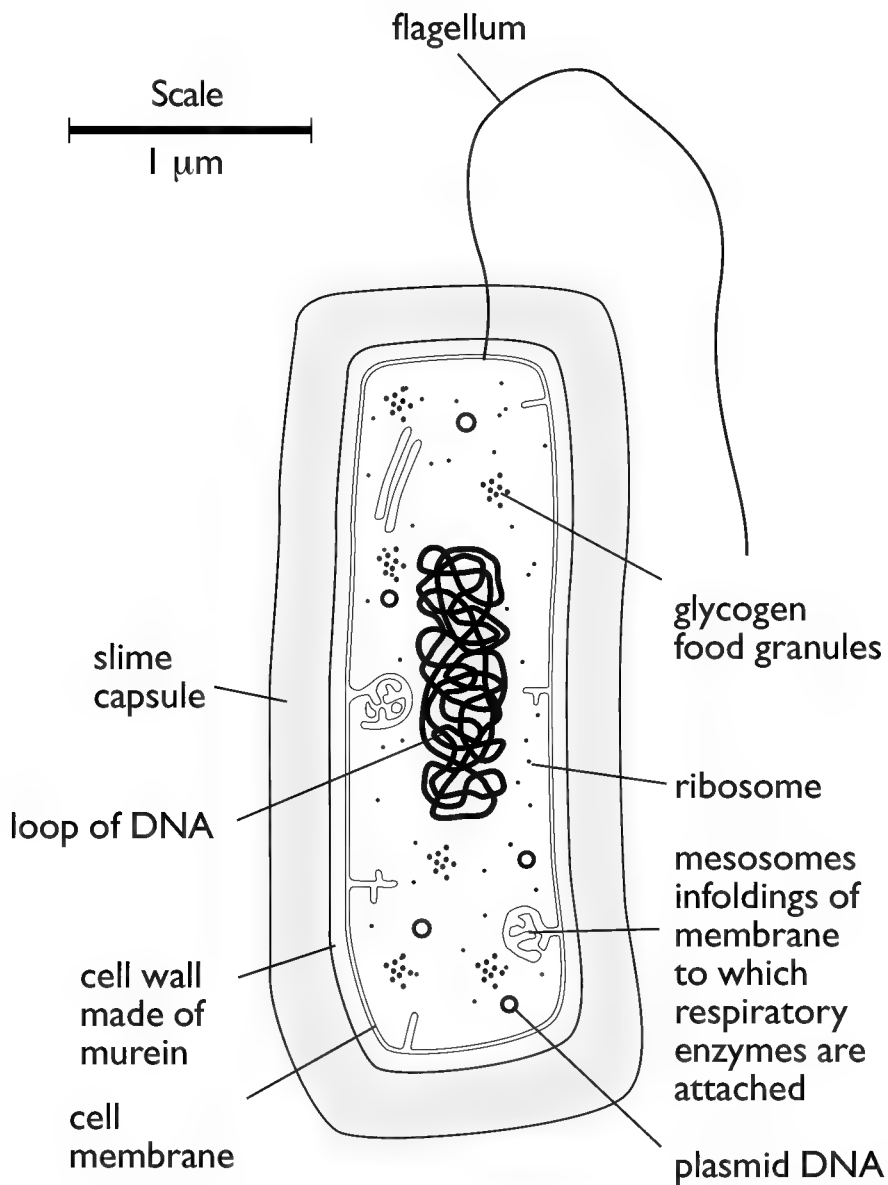
Resolution is the ability of an optical system to distinguish between adjacent points.

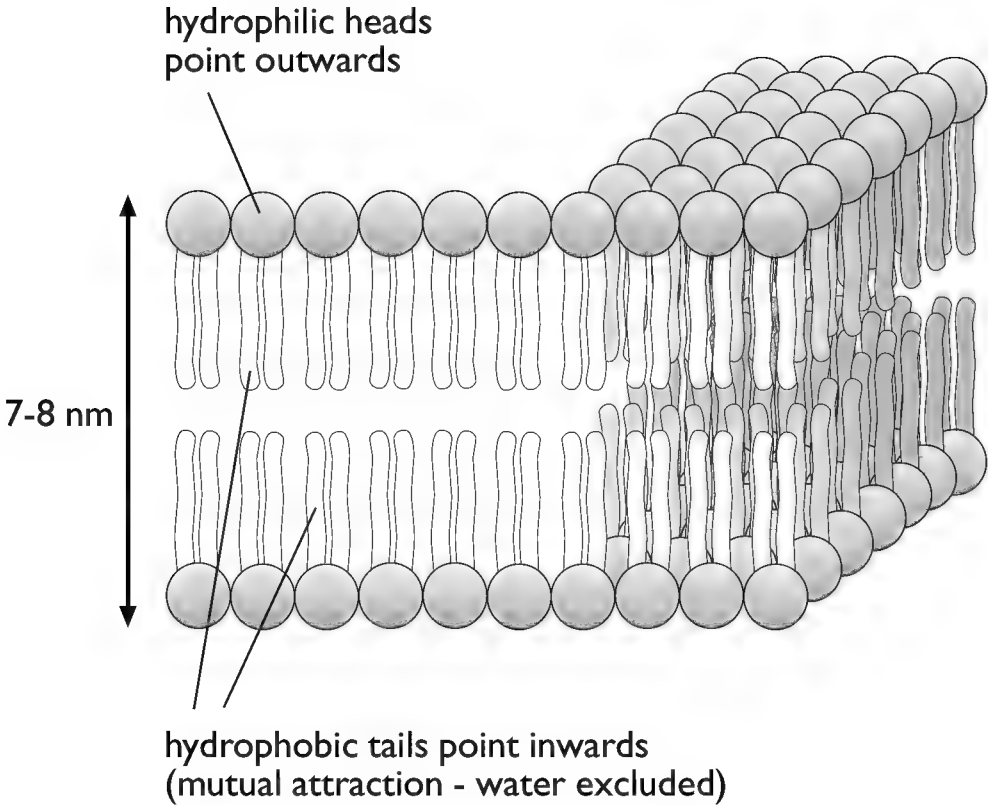


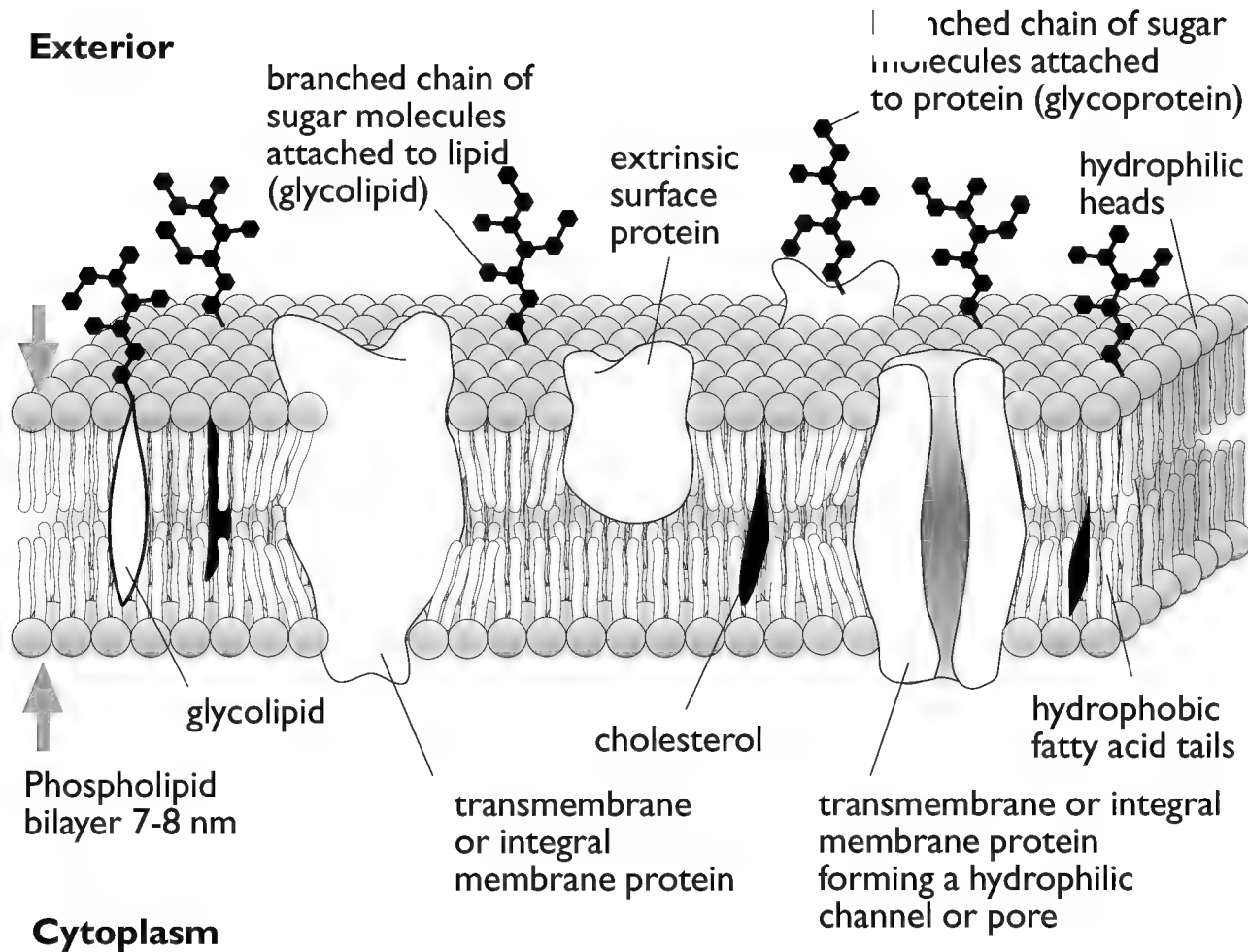


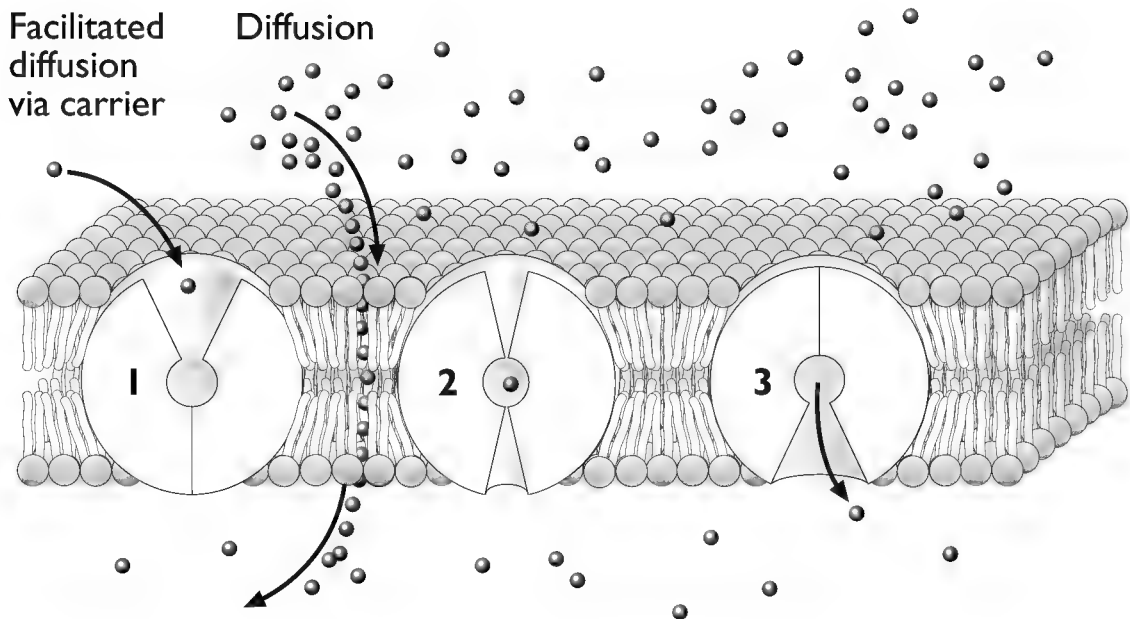


Structure of bacterium

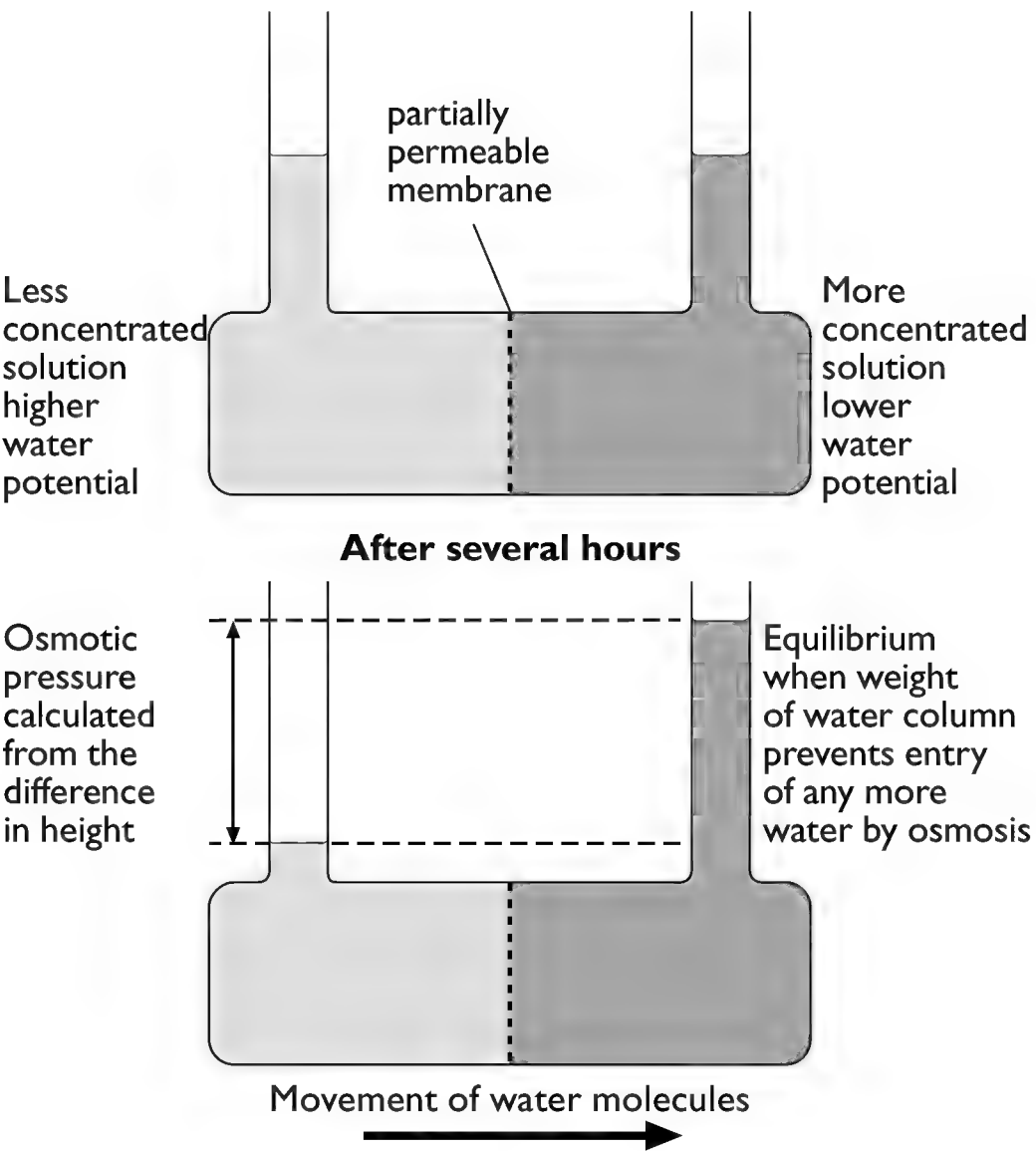




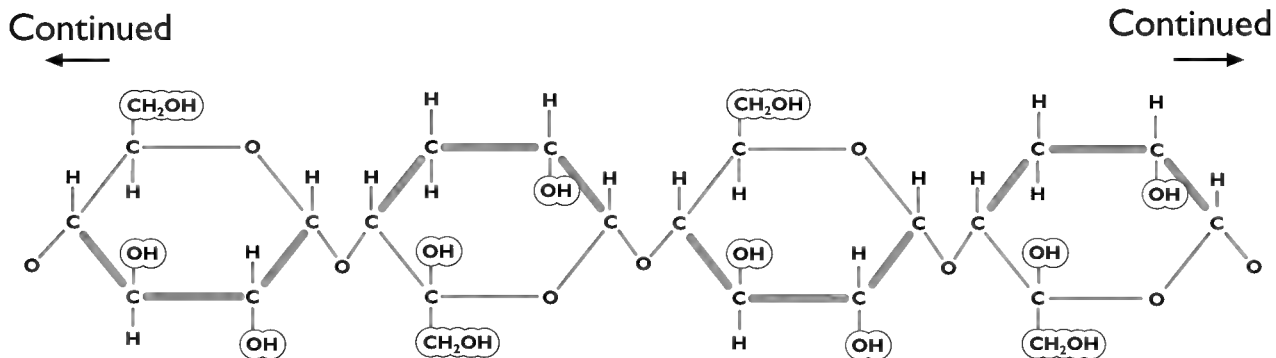




Water potential

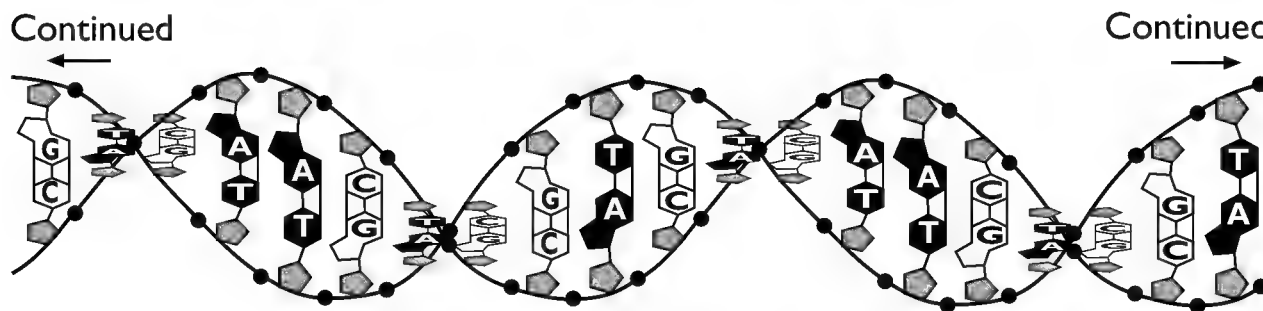


Part of a cellulose molecule chain

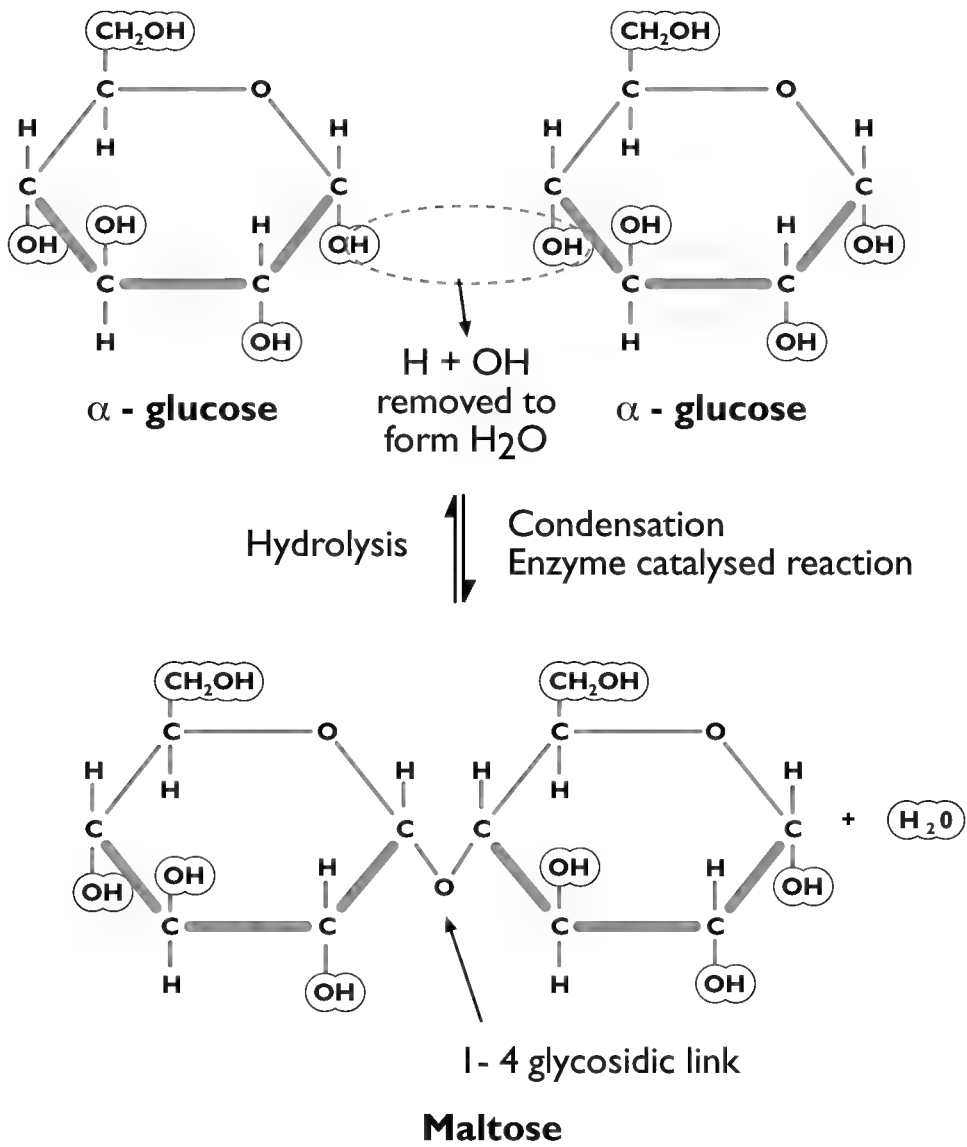


Notice how the CH_2OH groups alternate from side to side of the chain (the bonds indicated by a thick line are nearer)

Part of a DNA double helix

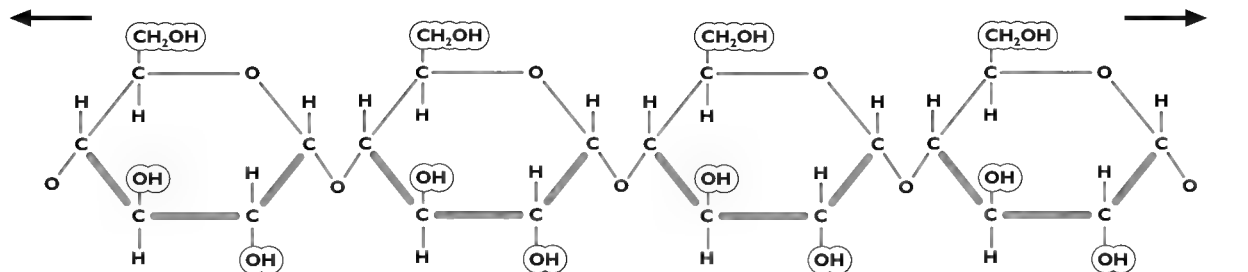


Monosaccharides and disaccharides

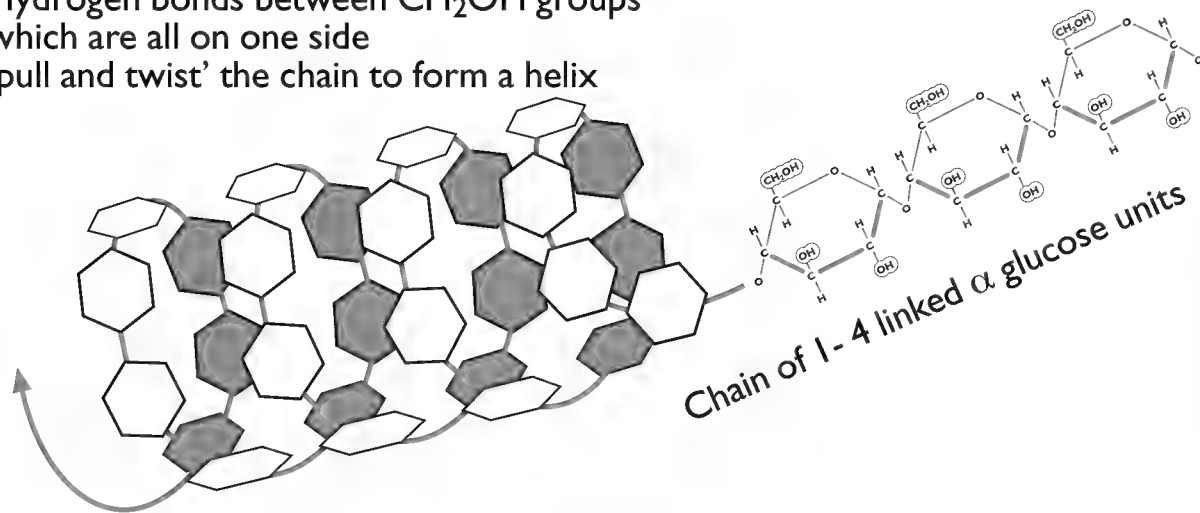


Part of amylose molecule chain

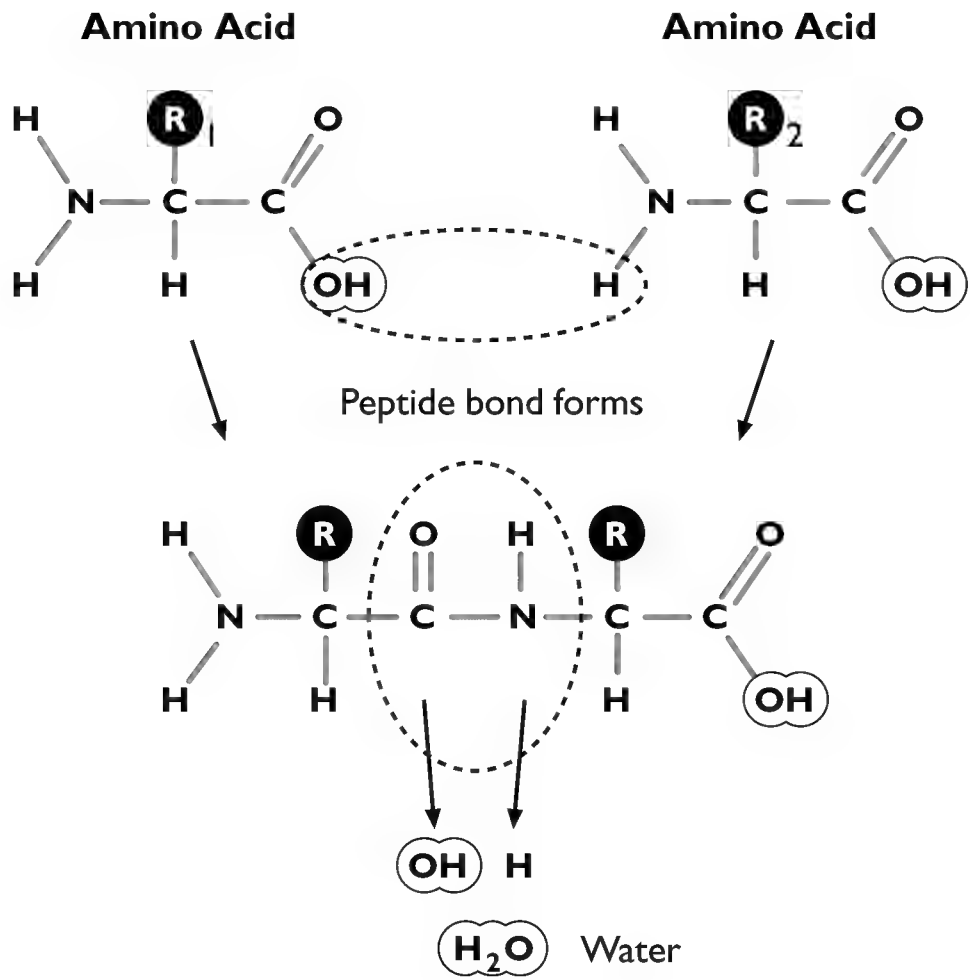
Continued



Hydrogen bonds between CH_2OH groups which are all on one side
'pull and twist' the chain to form a helix

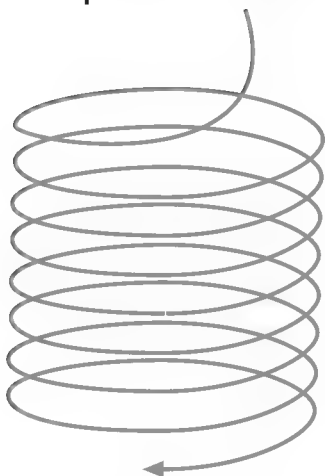


Amino acid structure and peptide bond

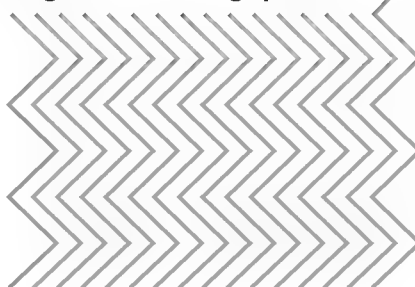


Secondary structure of proteins

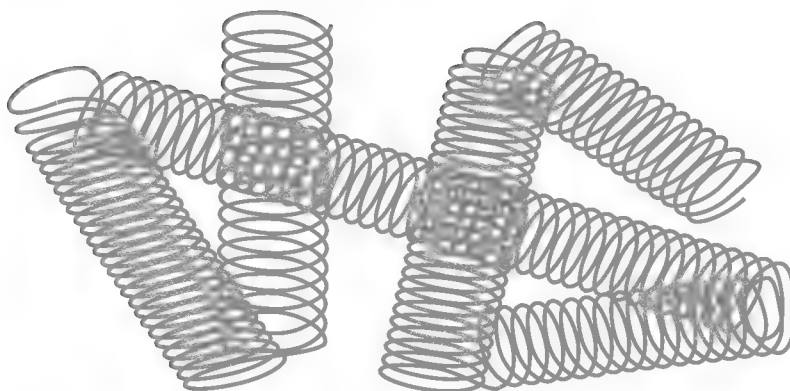
In an alpha helix hydrogen bonds 'pull' molecule chain into a spiral structure



In a beta sheet hydrogen bonds hold and align molecule chains side by side. Angle of bonds in each chain are as a consequence perfectly aligned forming 'pleats'.



Tertiary structure of proteins

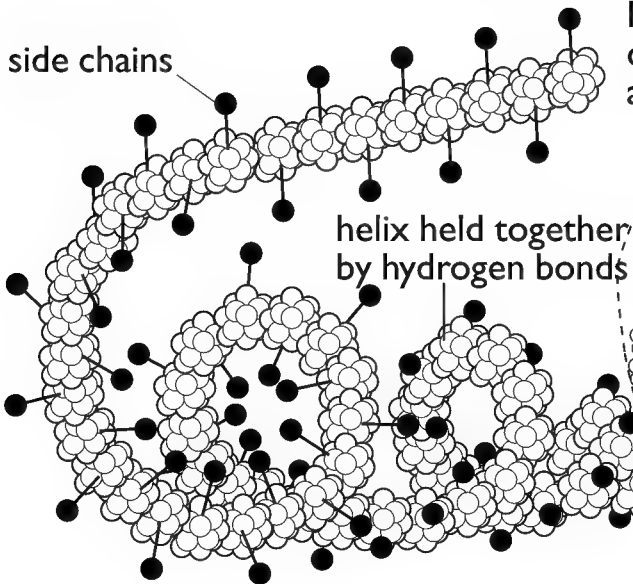


The alpha helix chain folds around itself forming a third level of structural organisation, just like a tangled telephone flex

Structure of haemoglobin

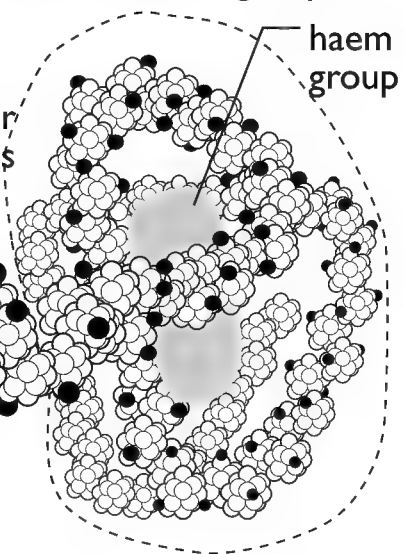
Primary structure

Amino acids join to form a polypeptide chain



Tertiary structure

Helix folds to form compact globular unit around Haem group



Secondary structure

Polypeptide chain folds in upon itself to form a helix

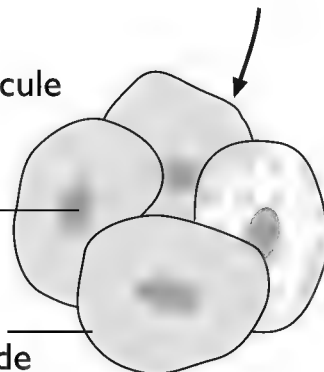
Haemoglobin molecule has four subunits

Quaternary structure

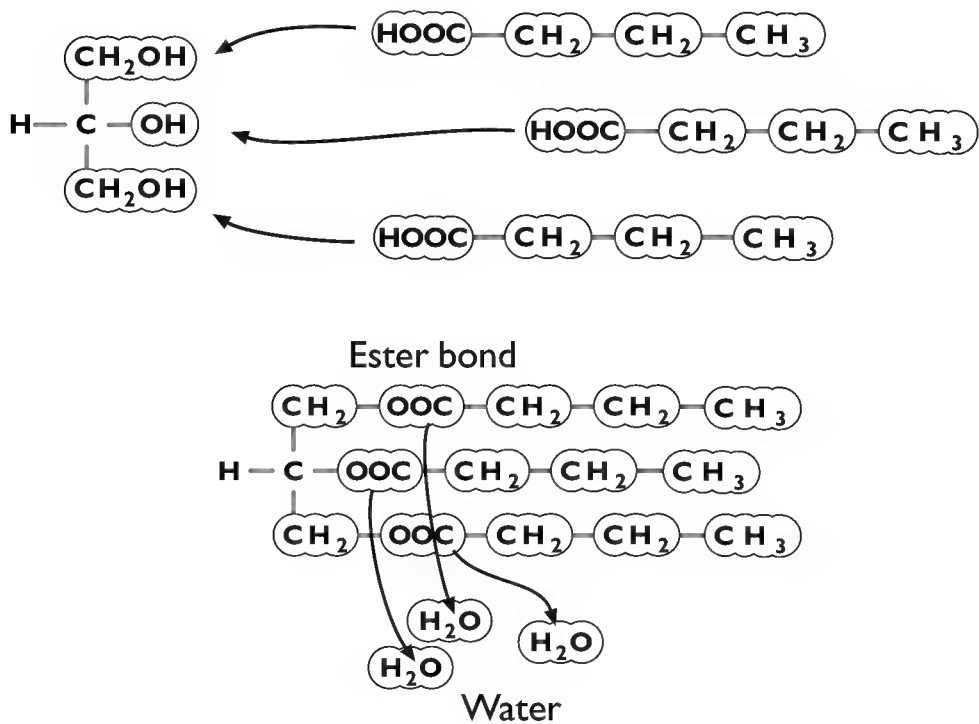
Several polypeptide chain units come together held by disulphide bridges to form a functional protein

haem group

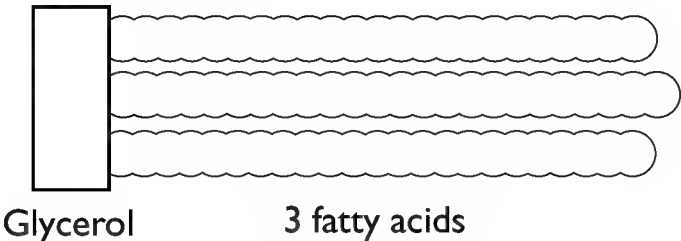
globular polypeptide subunit



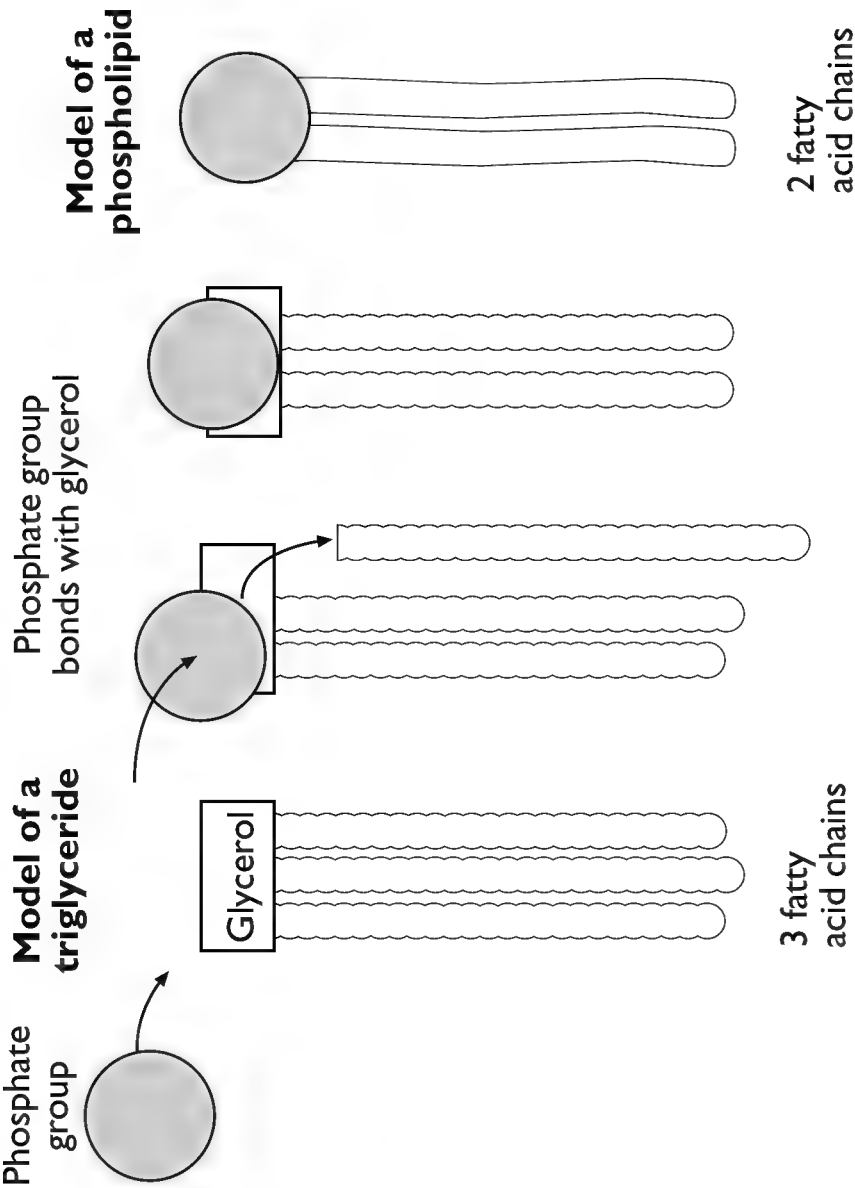
Structure of triglyceride



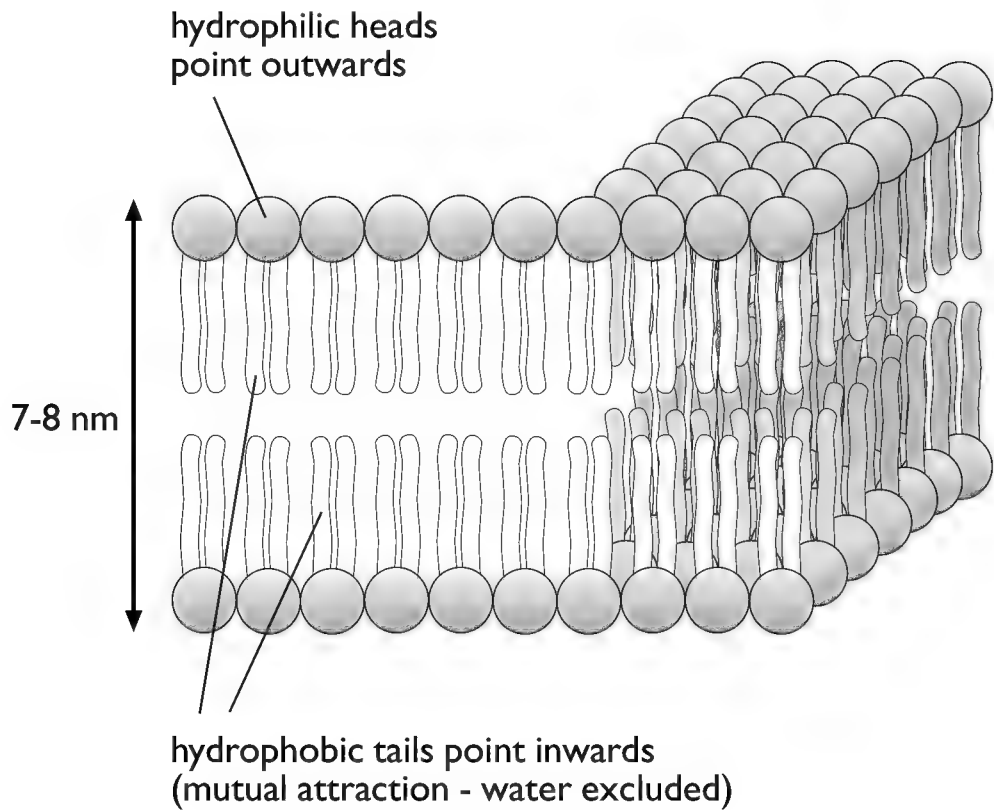
Model of a triglyceride

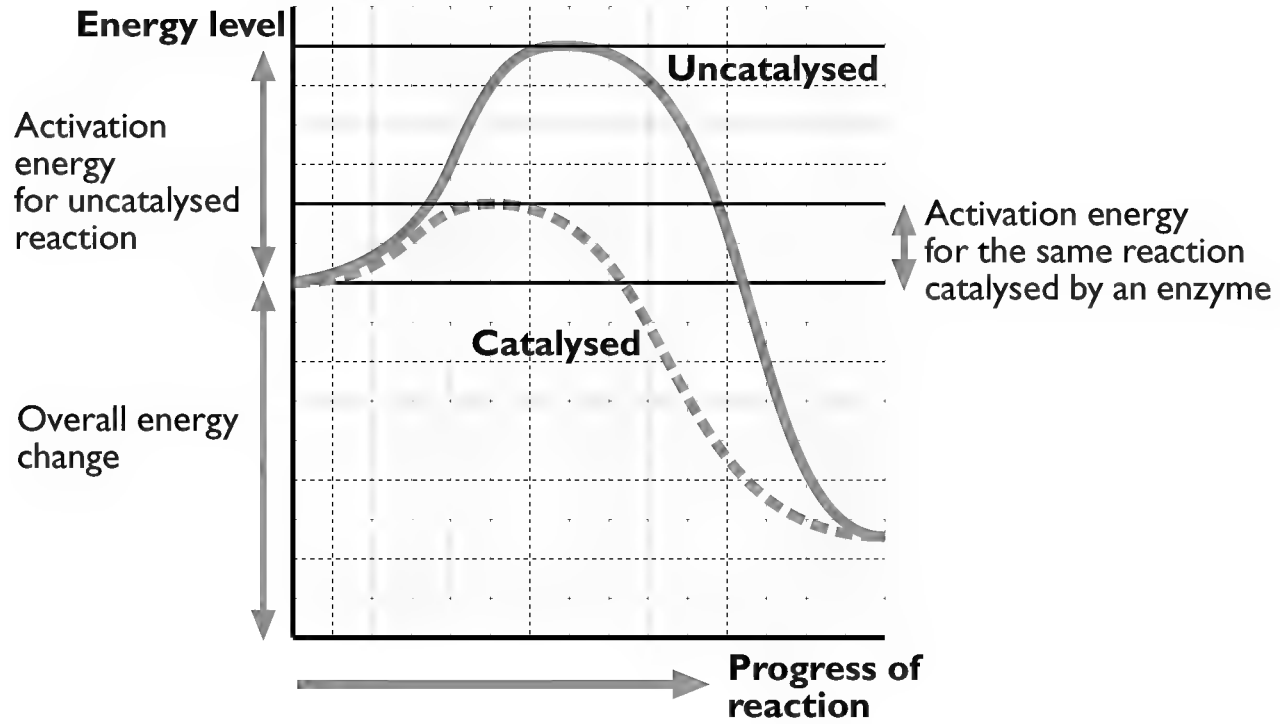


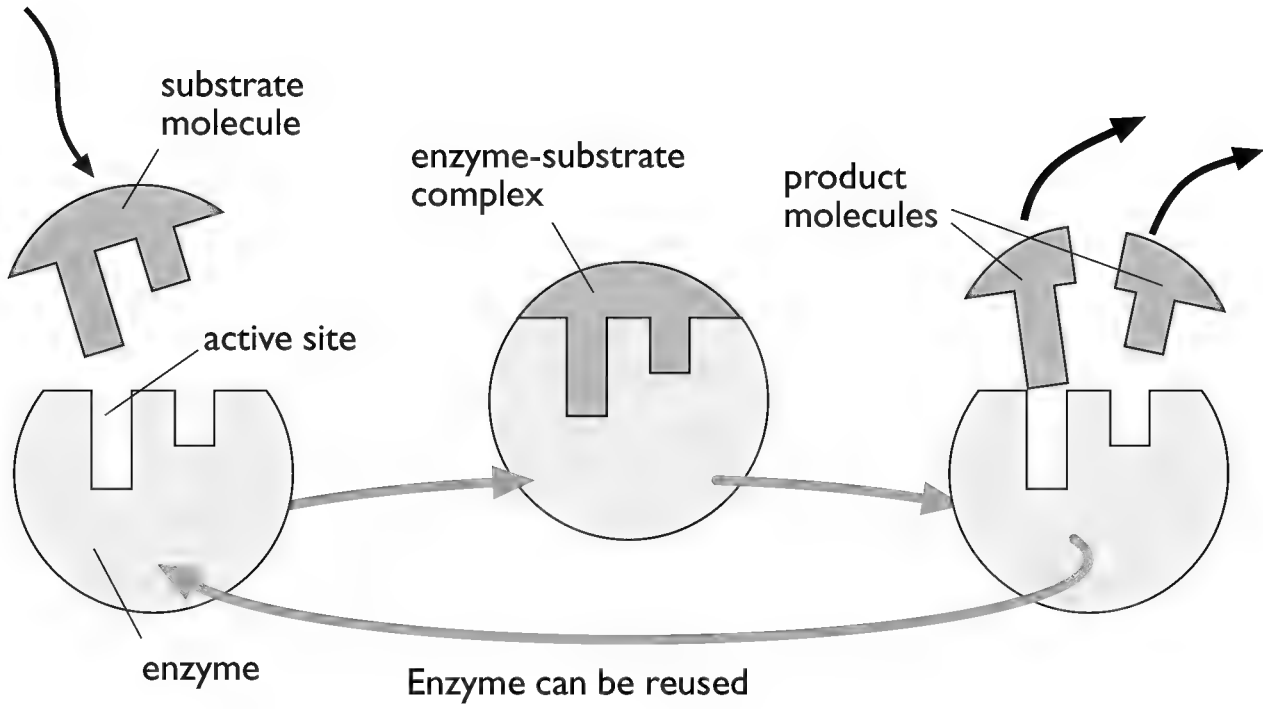
Structure of phospholipids



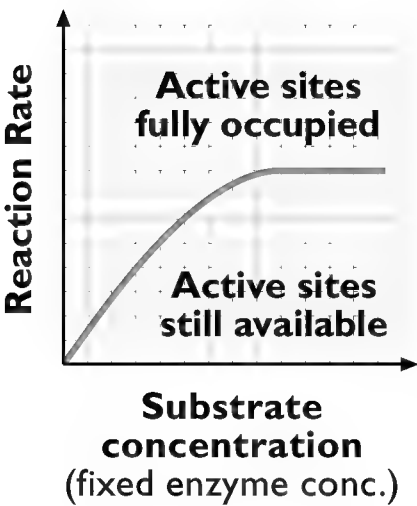
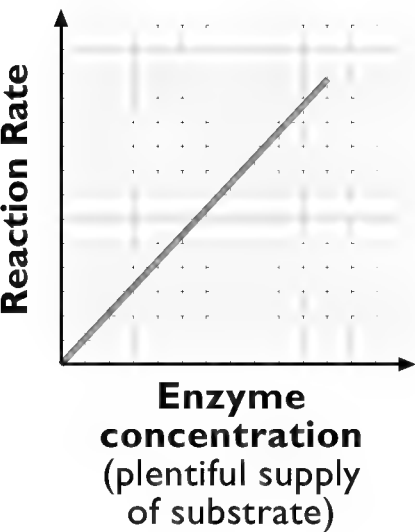
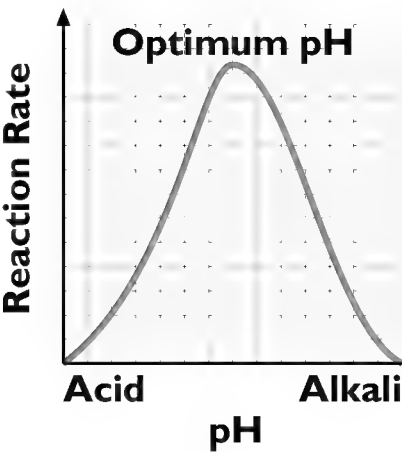
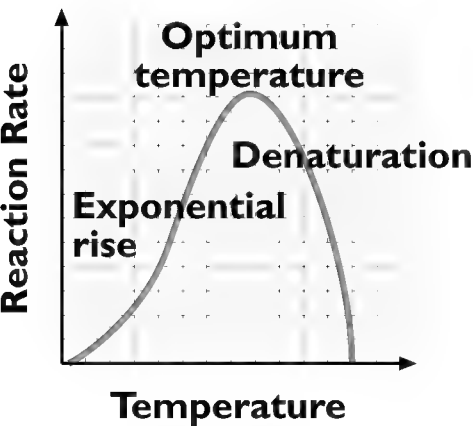
Phospholipids and the bilayer



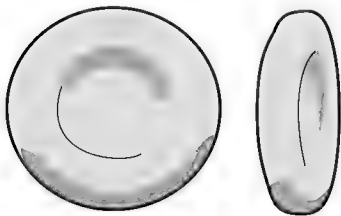




Temperature, pH, enzyme
and substrate concentration



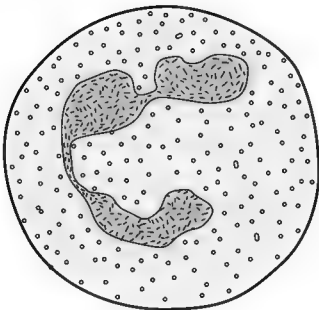
Blood components



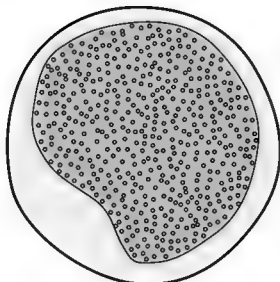
Red cell



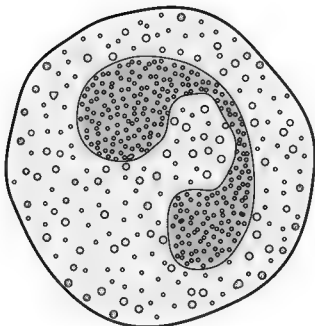
Platelets



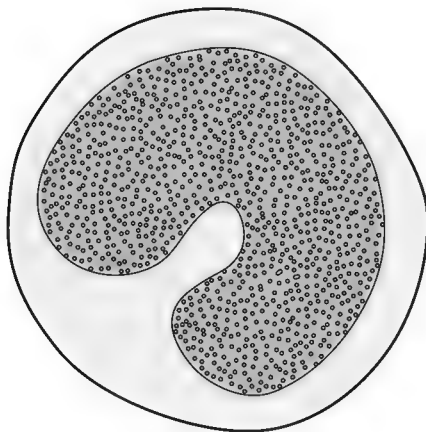
Neutrophil



Lymphocyte

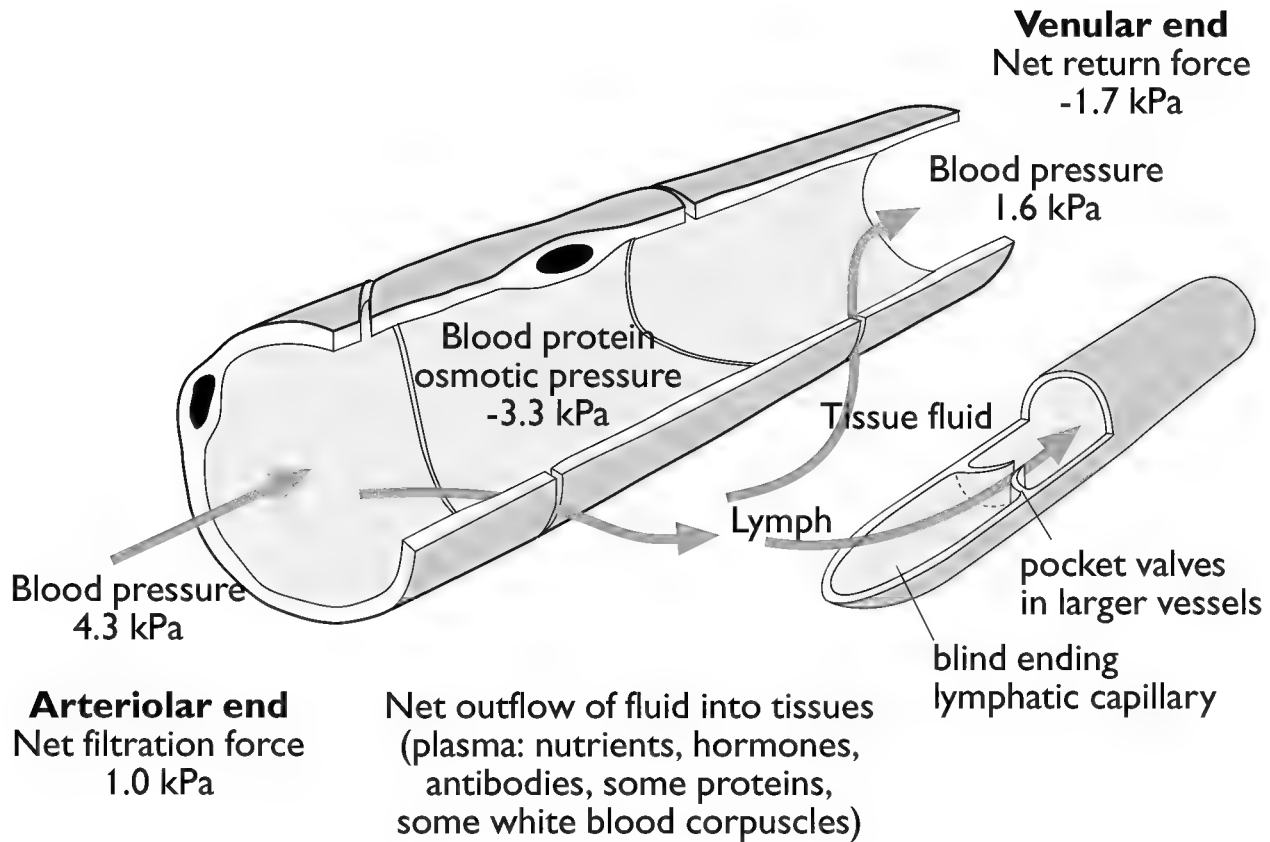


Eosinophil

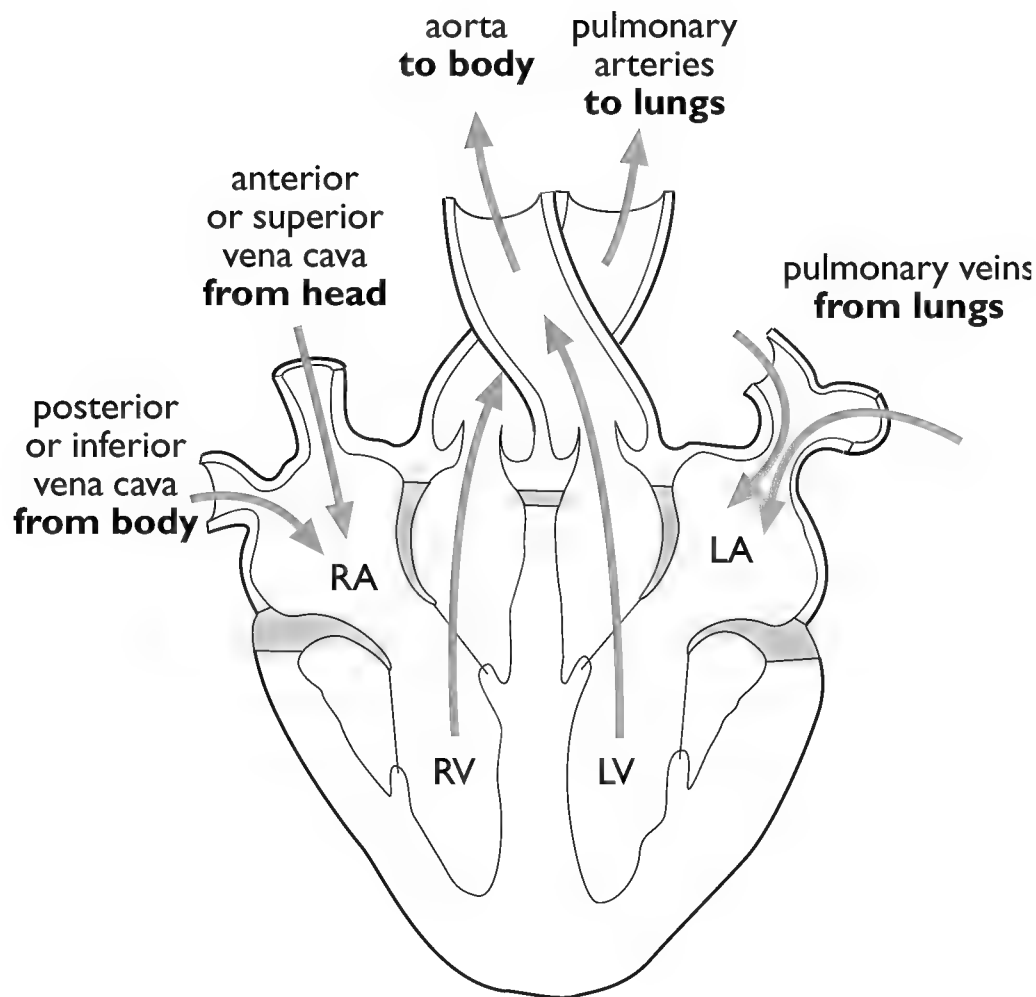


Monocyte

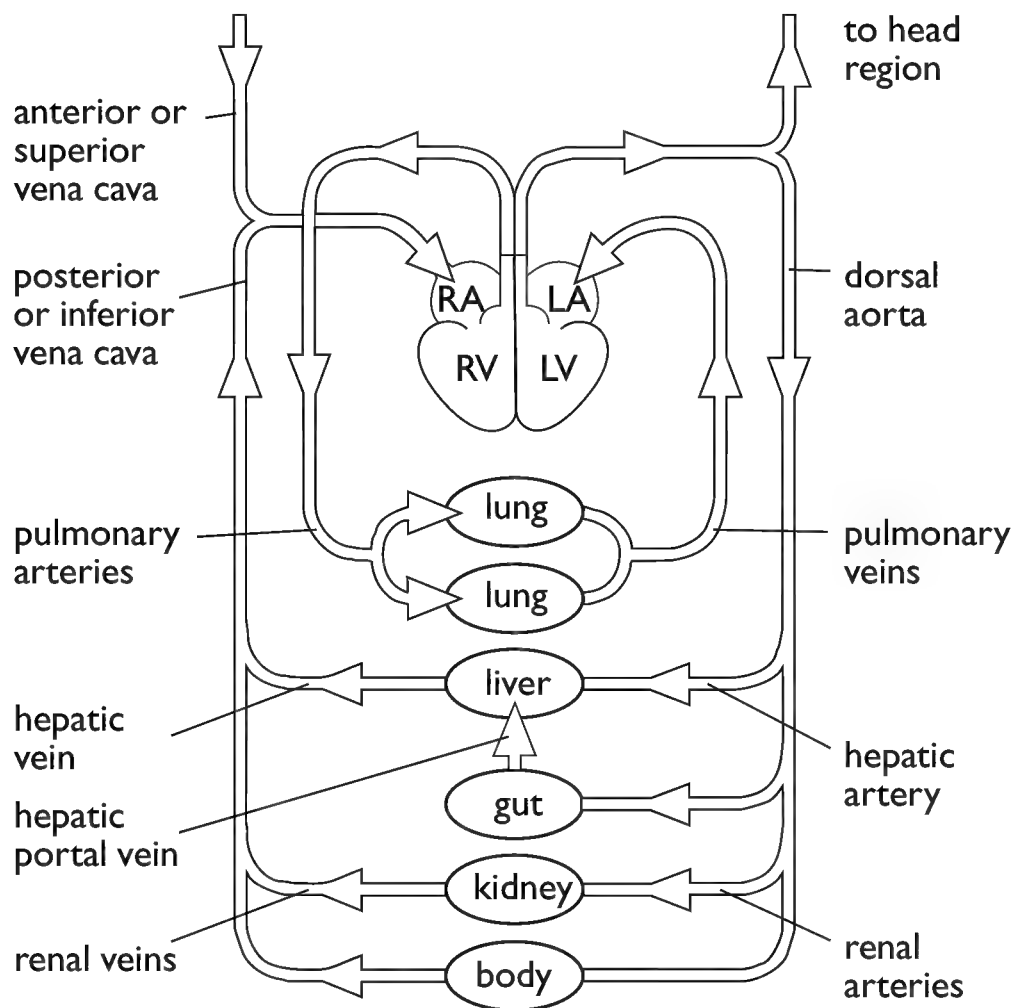
Diagram showing relationship between blood and lymph



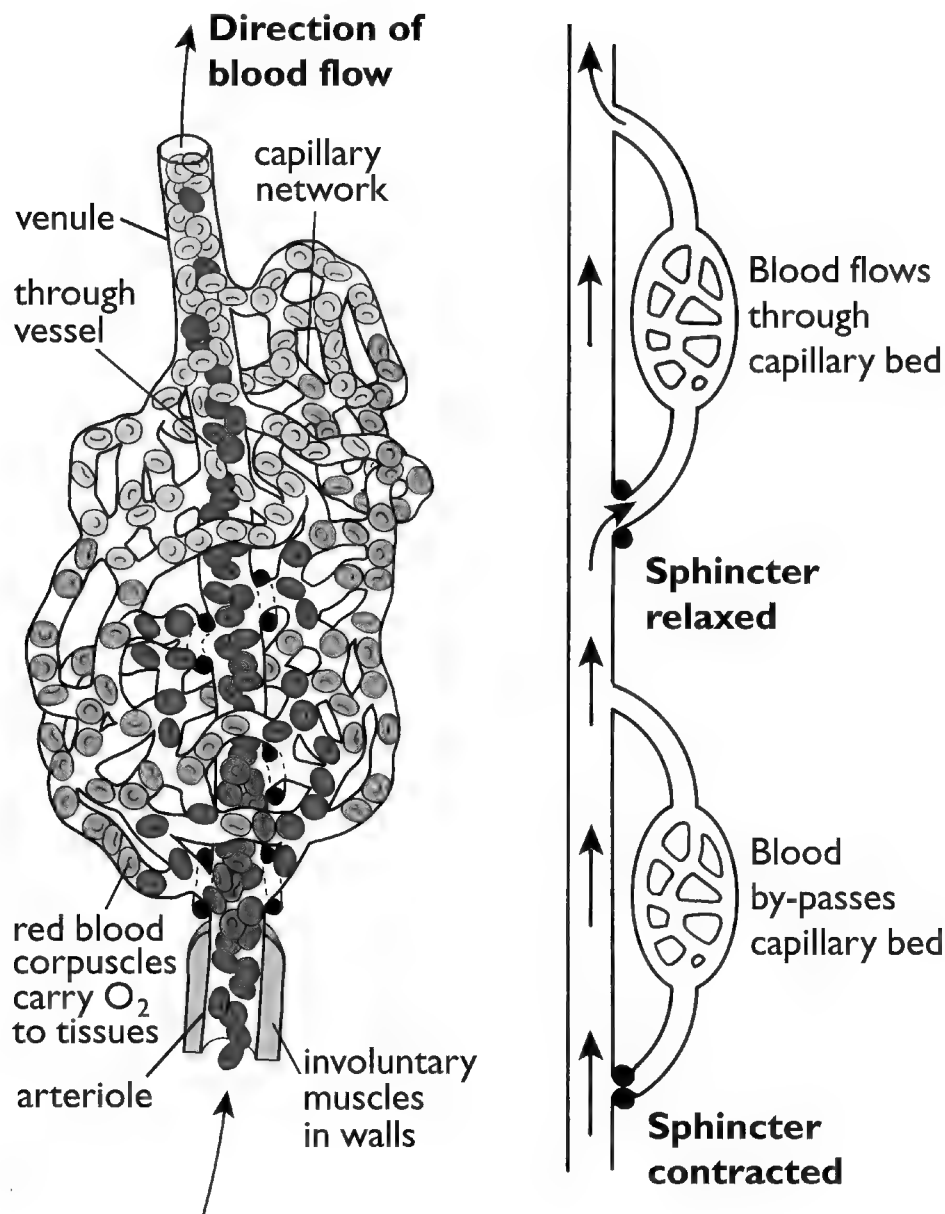
Mammalian heart



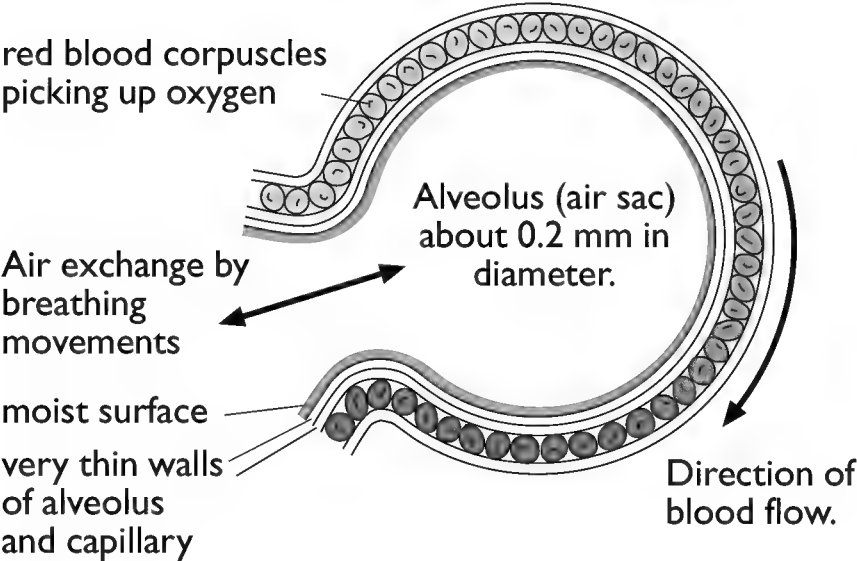
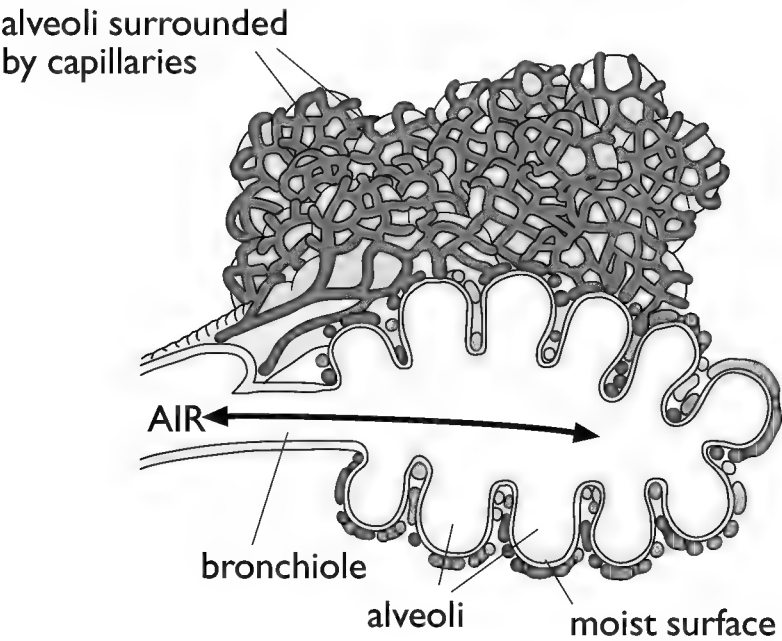
Closed double circulation diagram



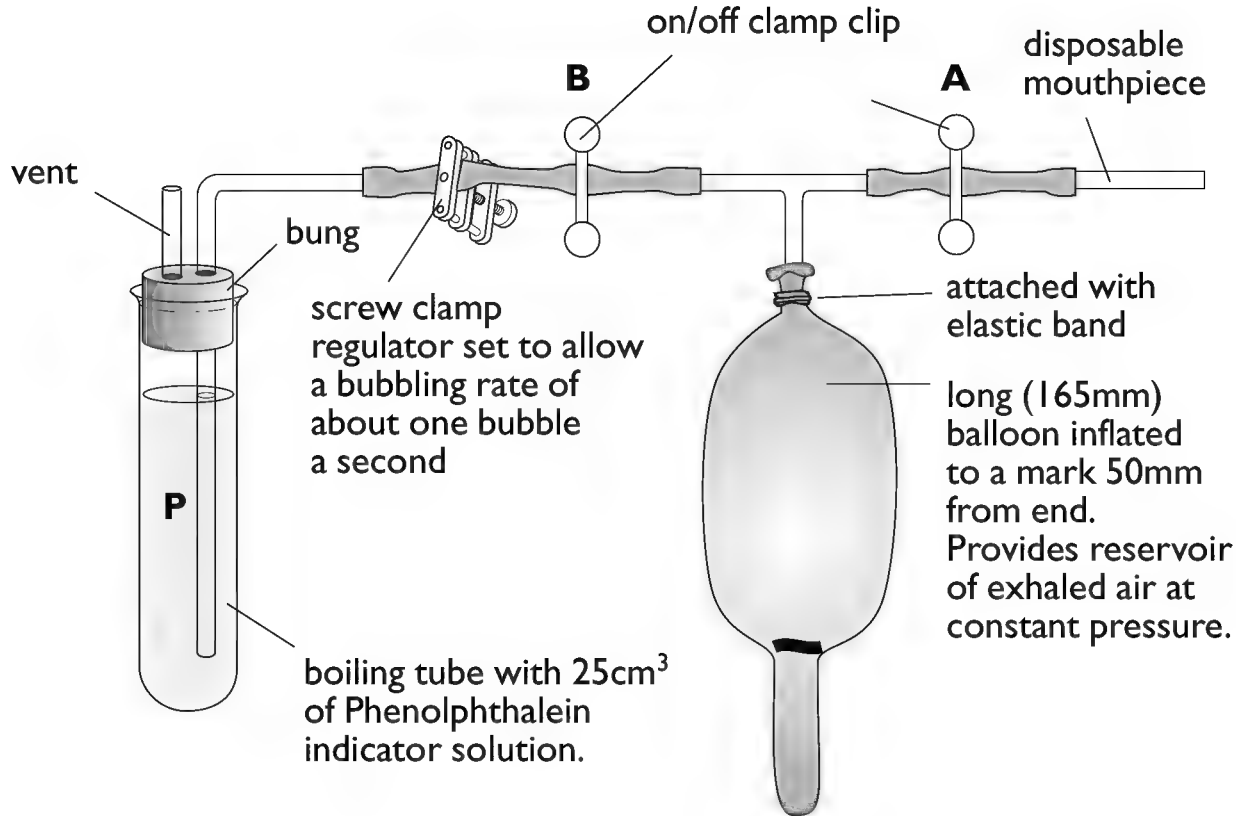
Capillary network

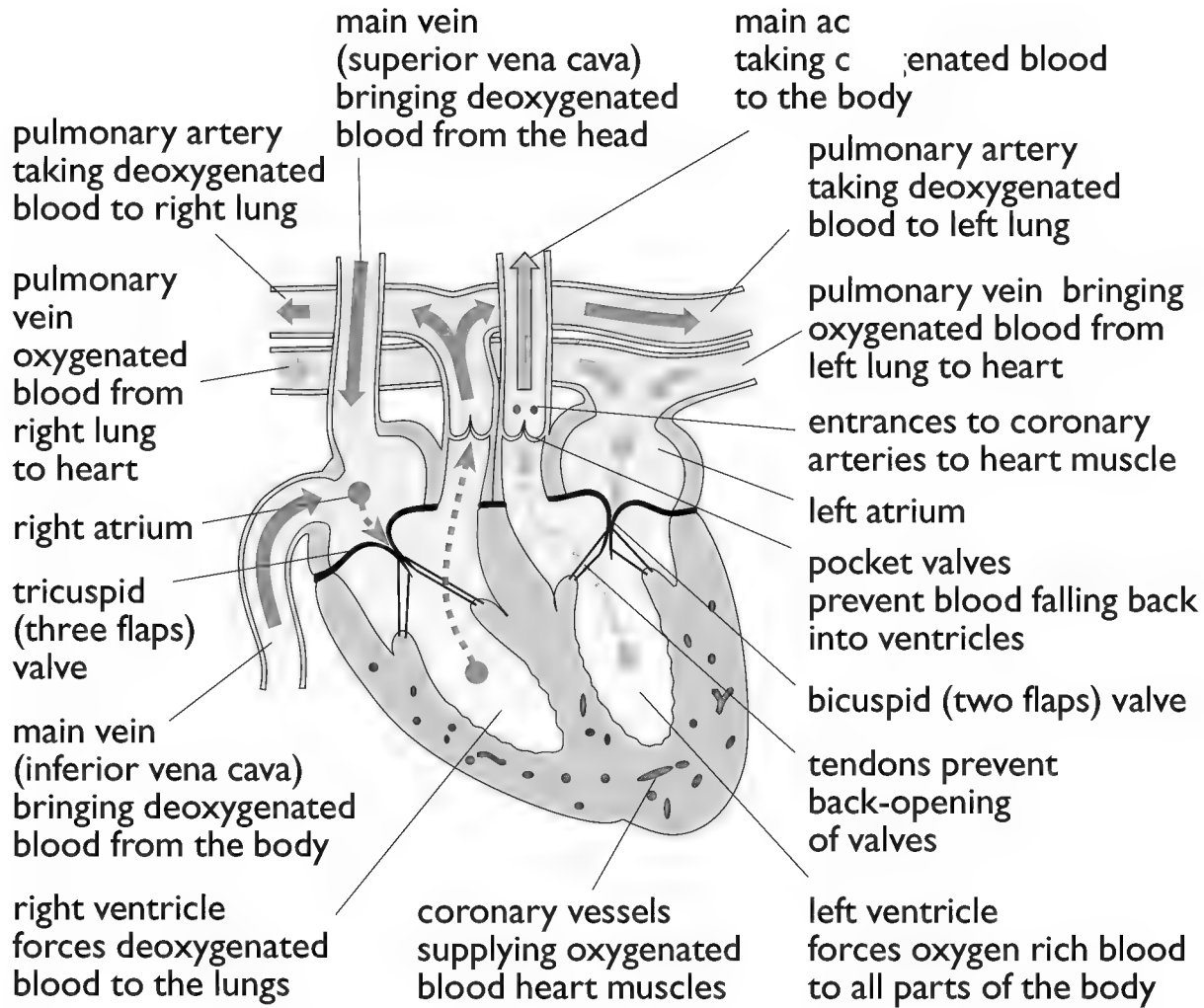


Alveoli and associated capillaries

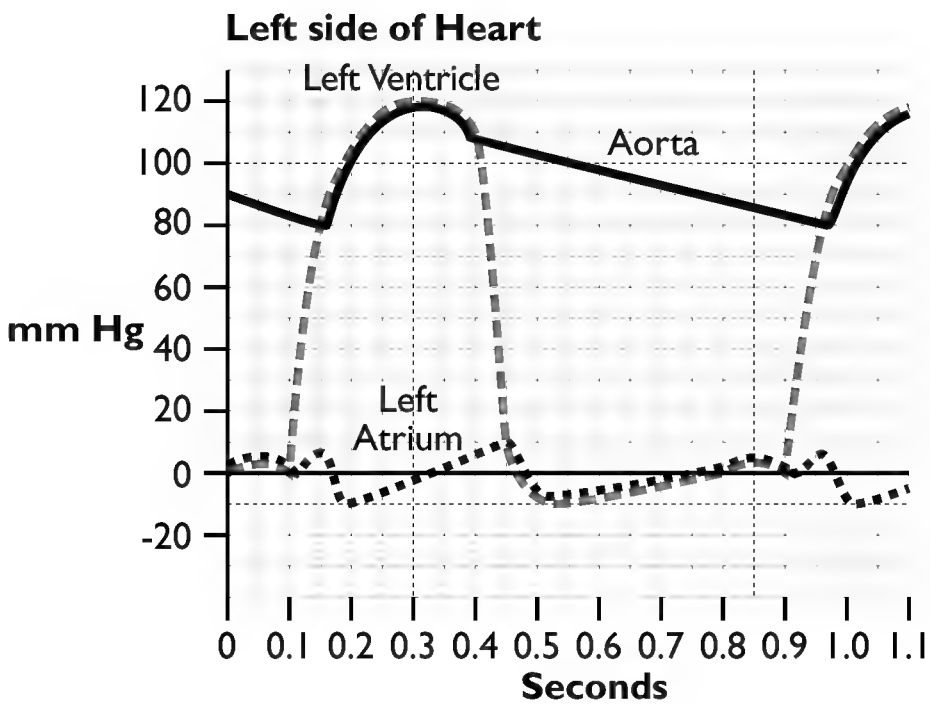
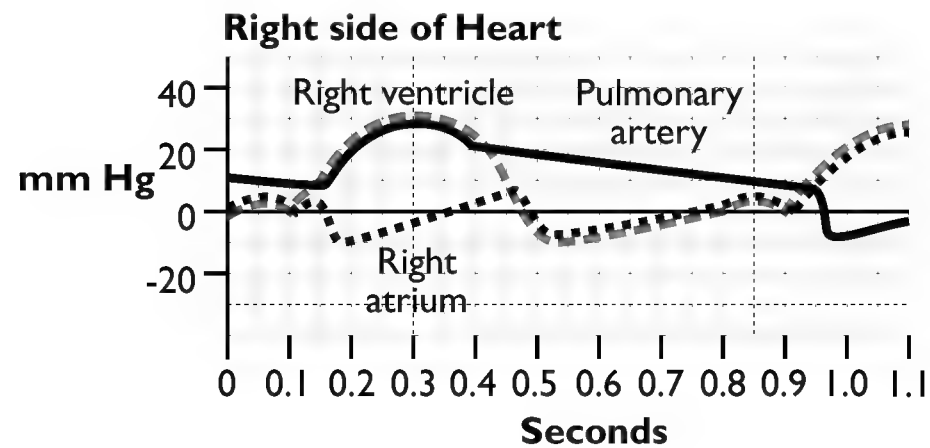


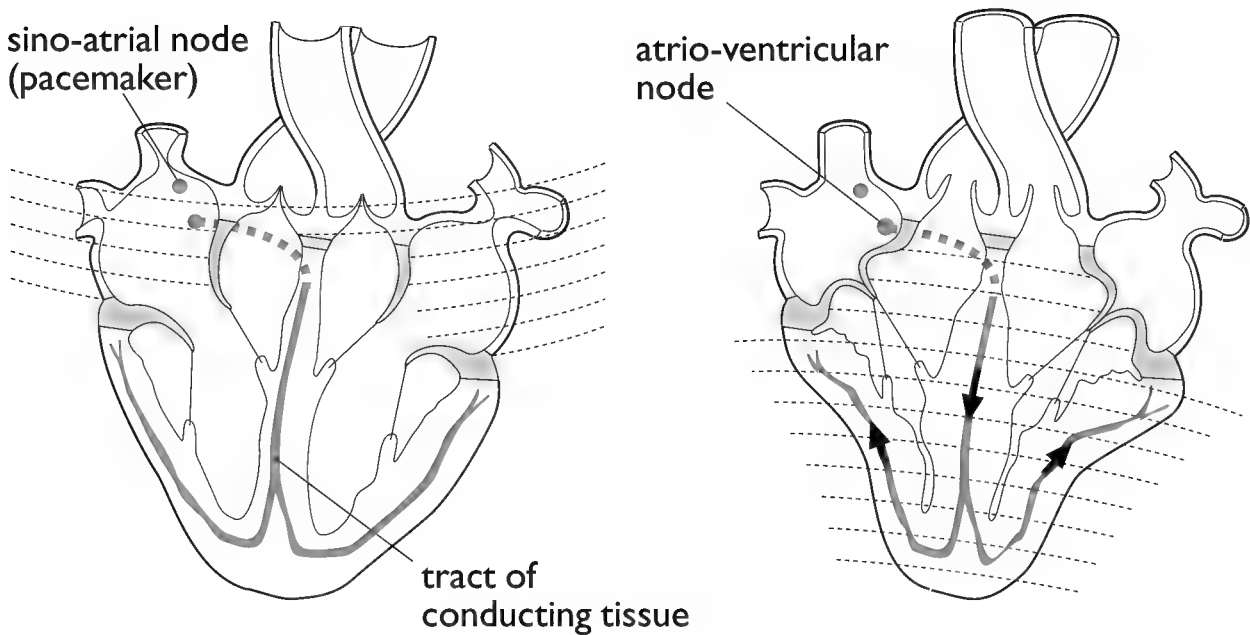
Simple apparatus for measurement of carbon dioxide levels in expired air



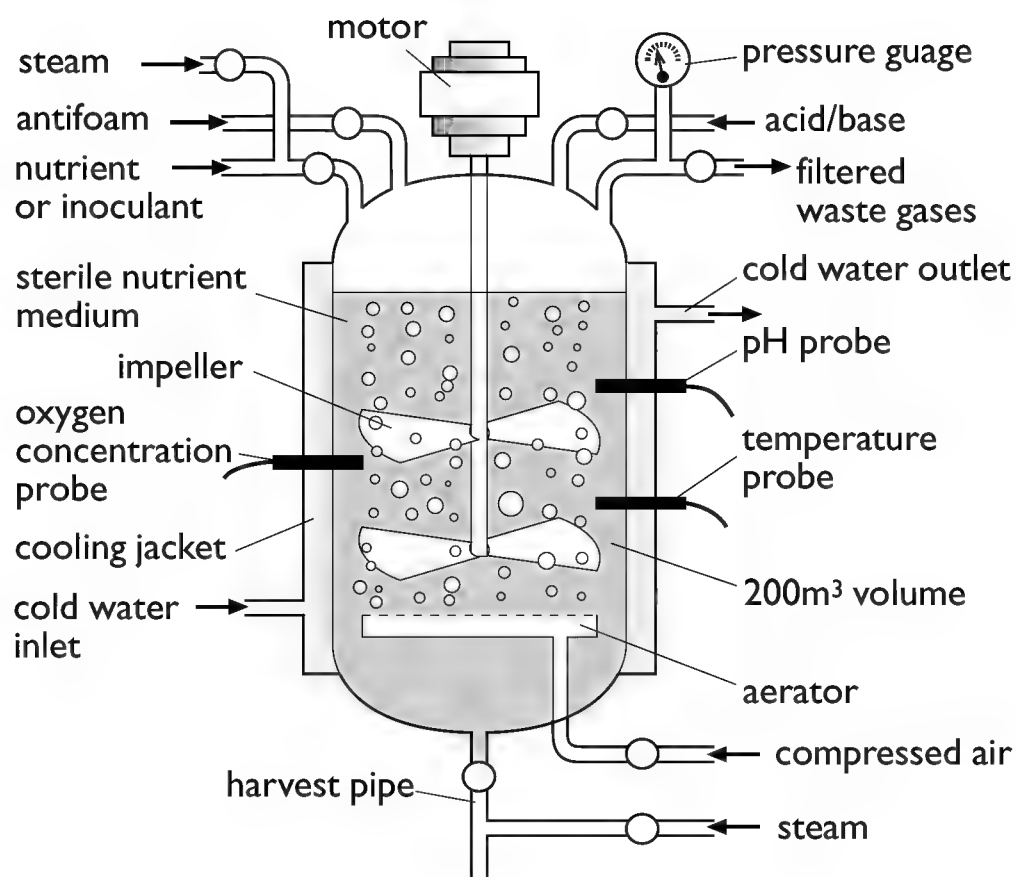


Graph/chart of pressure changes during the cardiac cycle



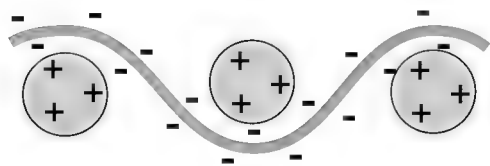


Fermenter

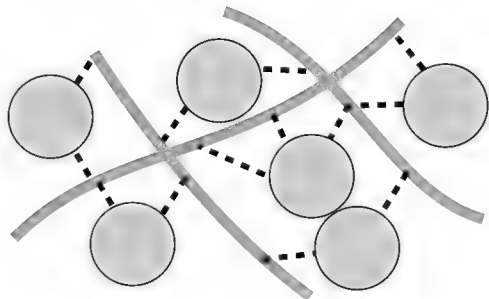


Immobilisation of enzymes

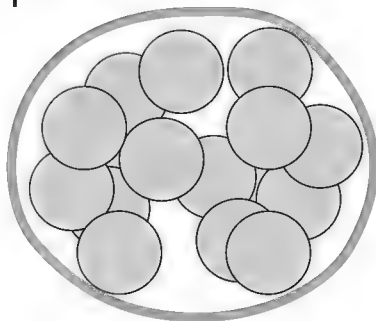
Absorbption



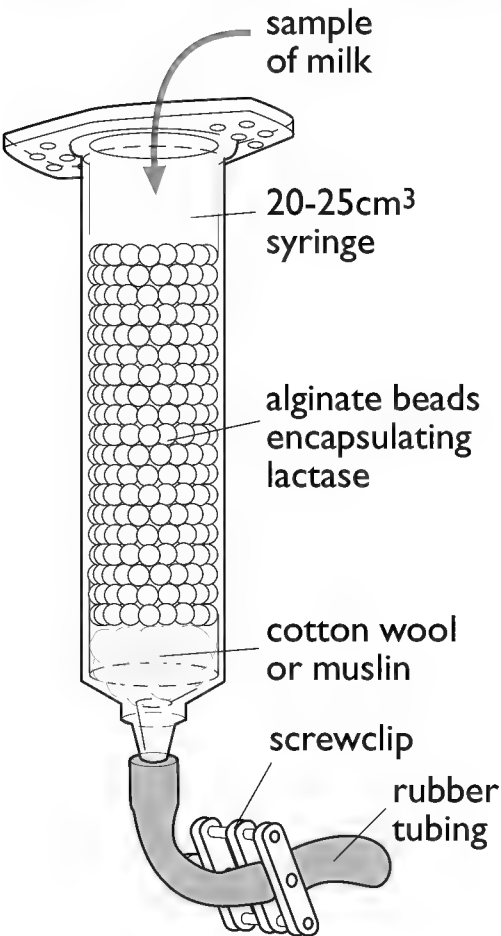
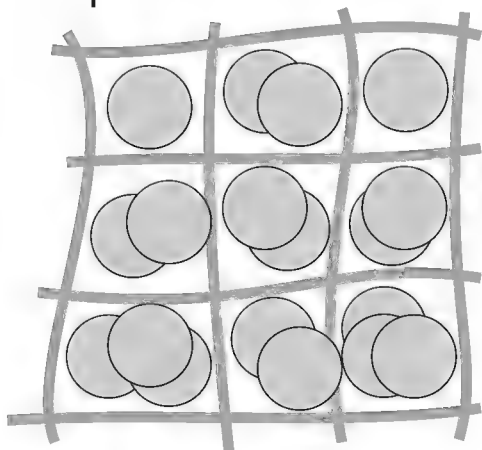
Covalent binding

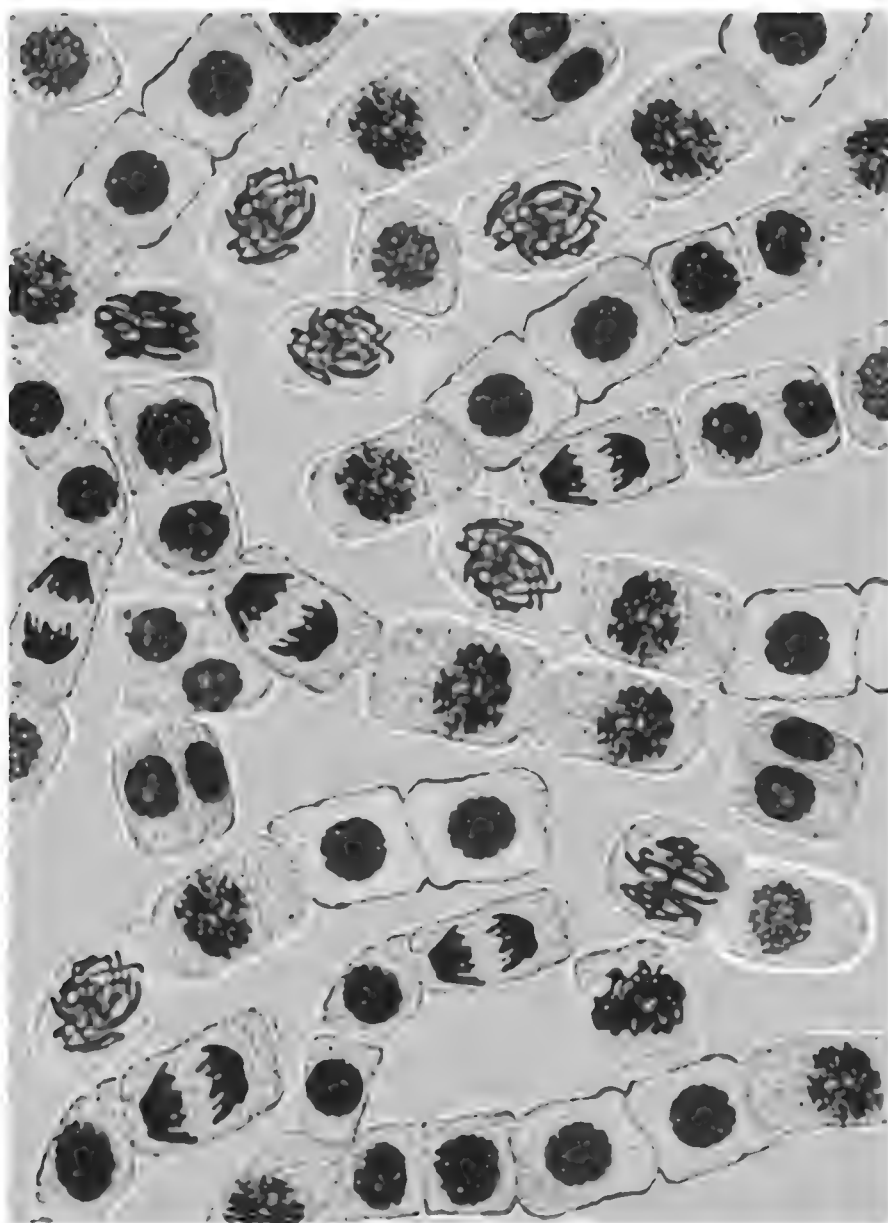


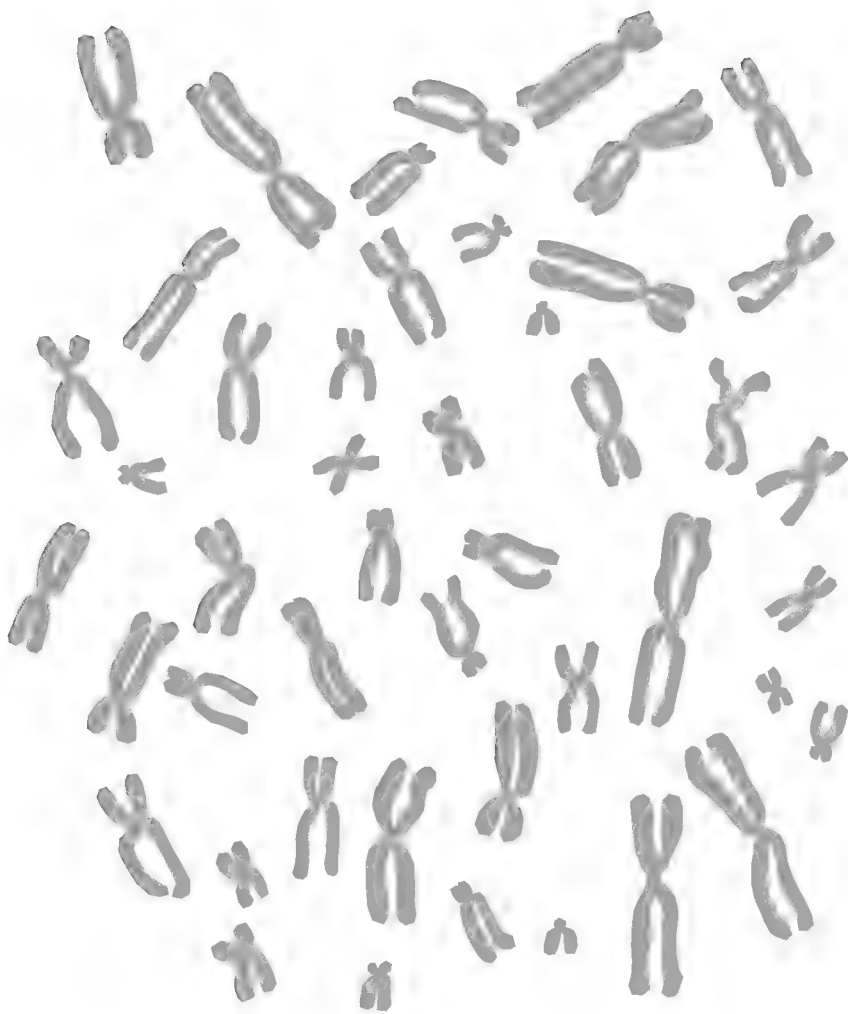
Encapsulation



Entrapment

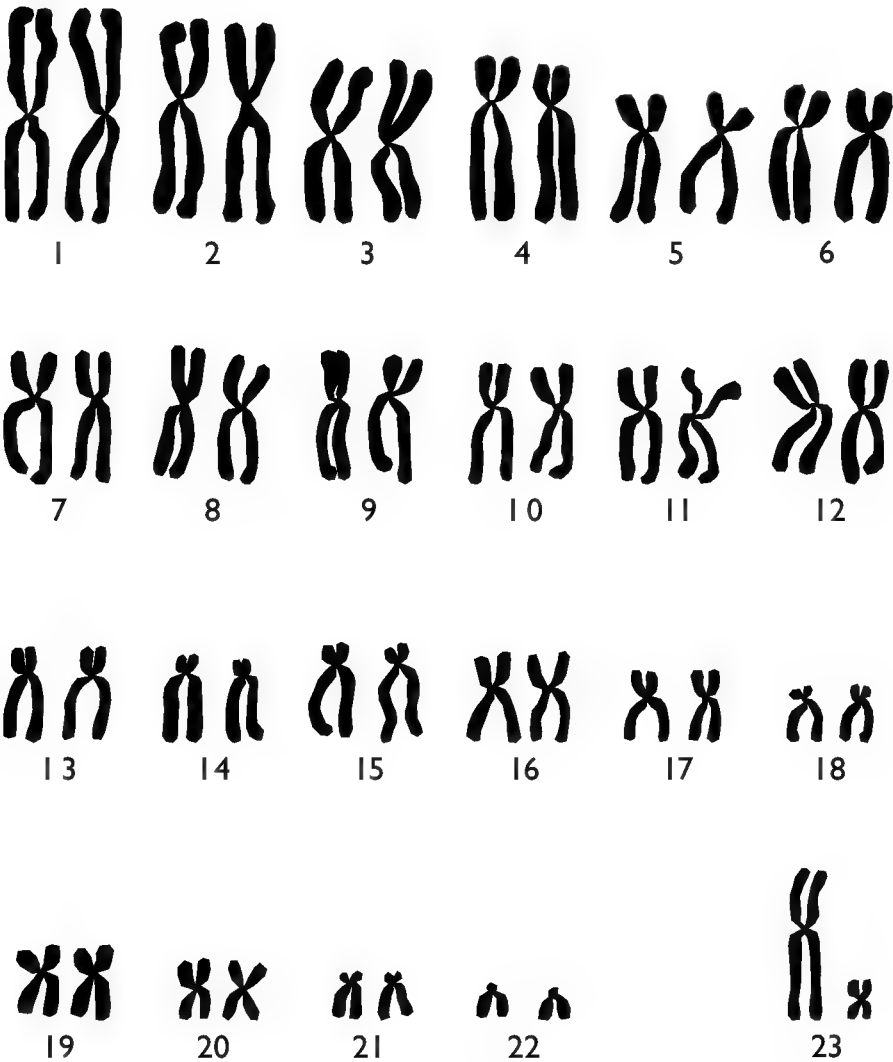




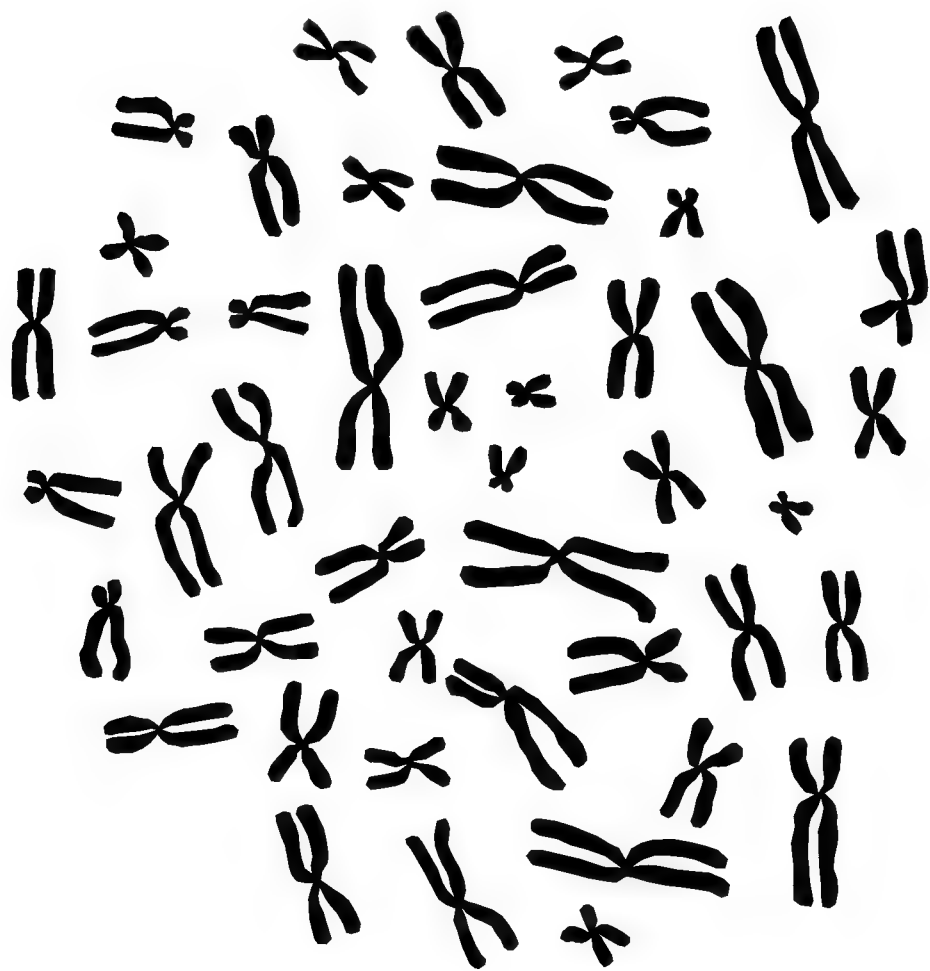


Human karyotype analysed into homologous pairs

Normal Human Male

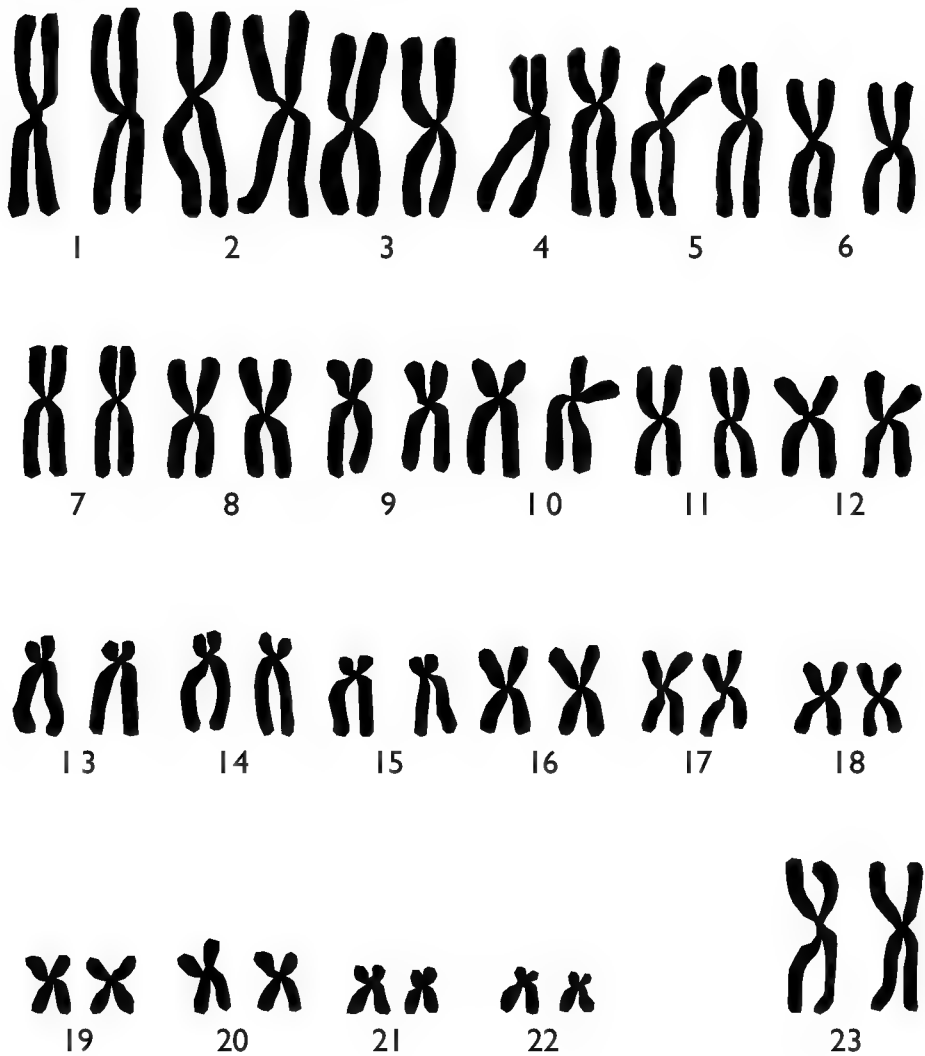


Normal human karyotype - B



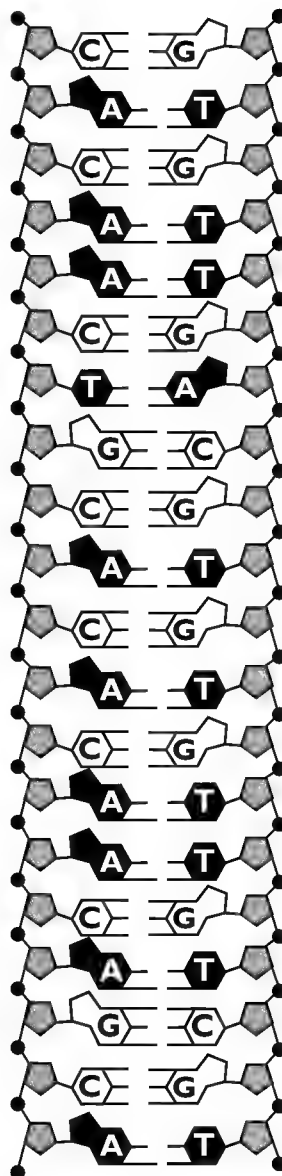
Human karyotype
analysed into homologous pairs

Normal Human Female



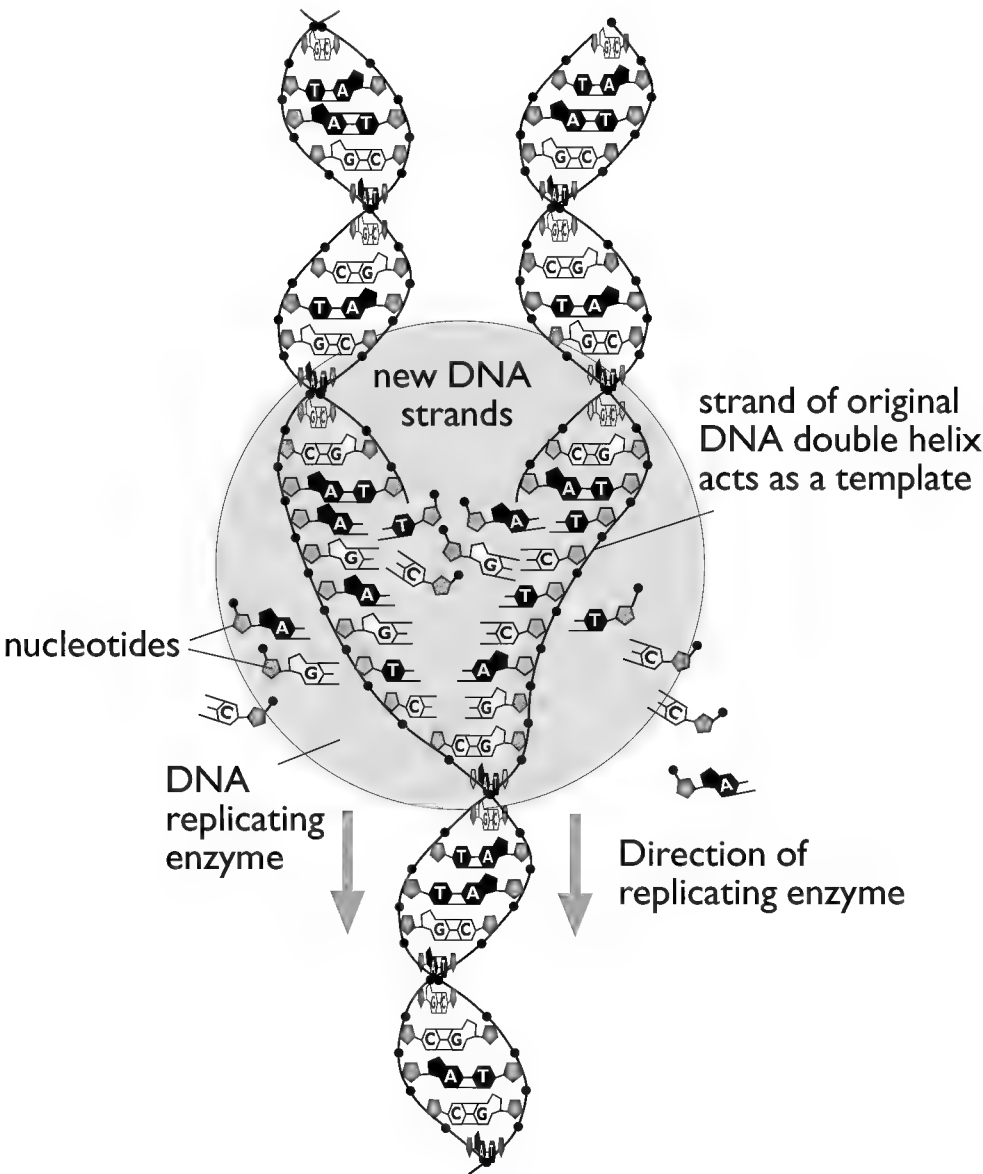
911

The diagram illustrates the structure of a DNA double helix. The sugar-phosphate backbone is shown as a series of alternating deoxyribose sugar molecules (pentagons) and phosphate groups (circles labeled 'P'). The nitrogenous bases are represented by polygons: Adenine (black), Guanine (white), Cytosine (white), and Thymine (black). The bases are paired with each other, forming the rungs of the double helix.



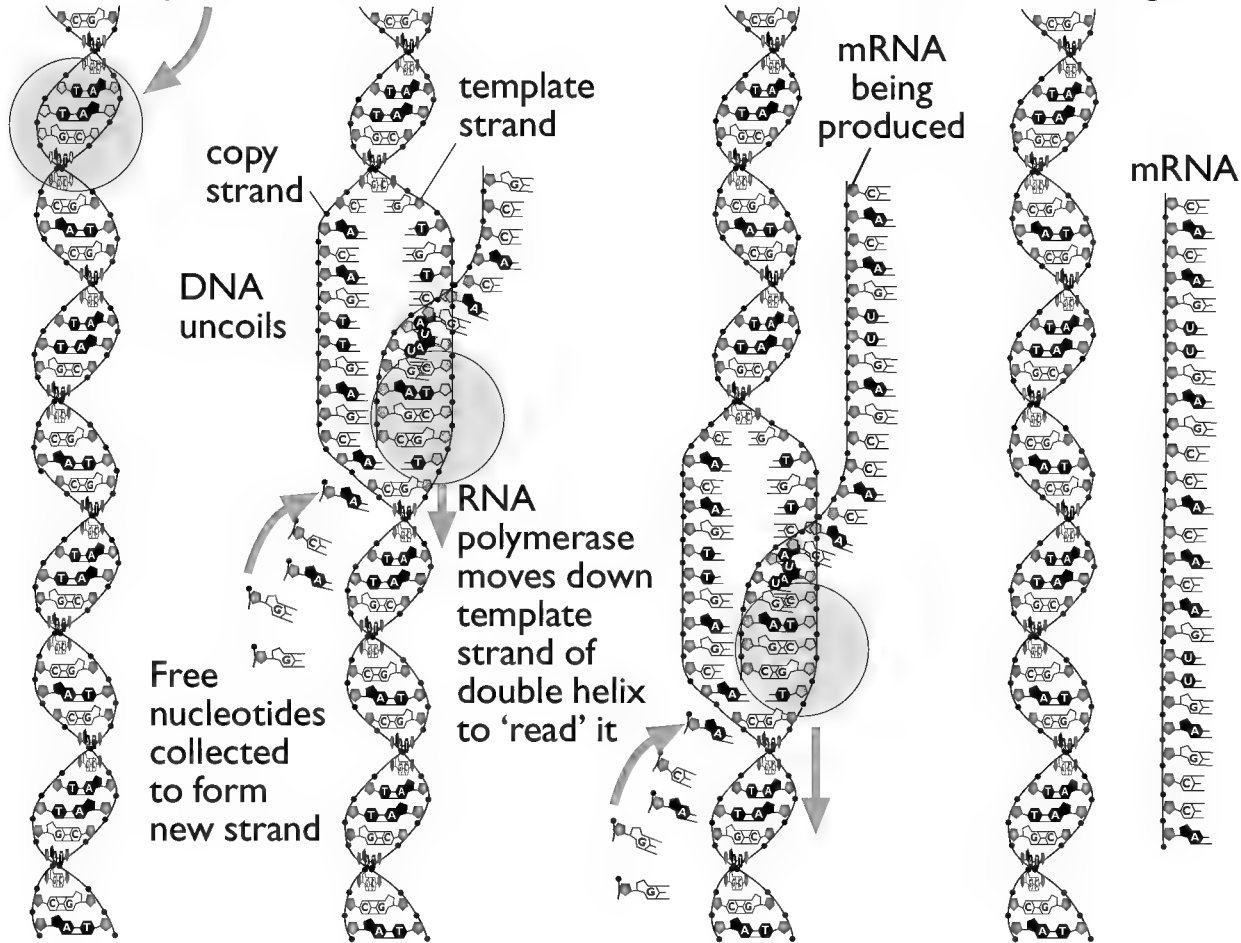
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Replication of DNA



RNA polymerase attaches to DNA strand at specific codon site

DNA double helix rewinds to original



Translation of mRNA code to build a polypeptide

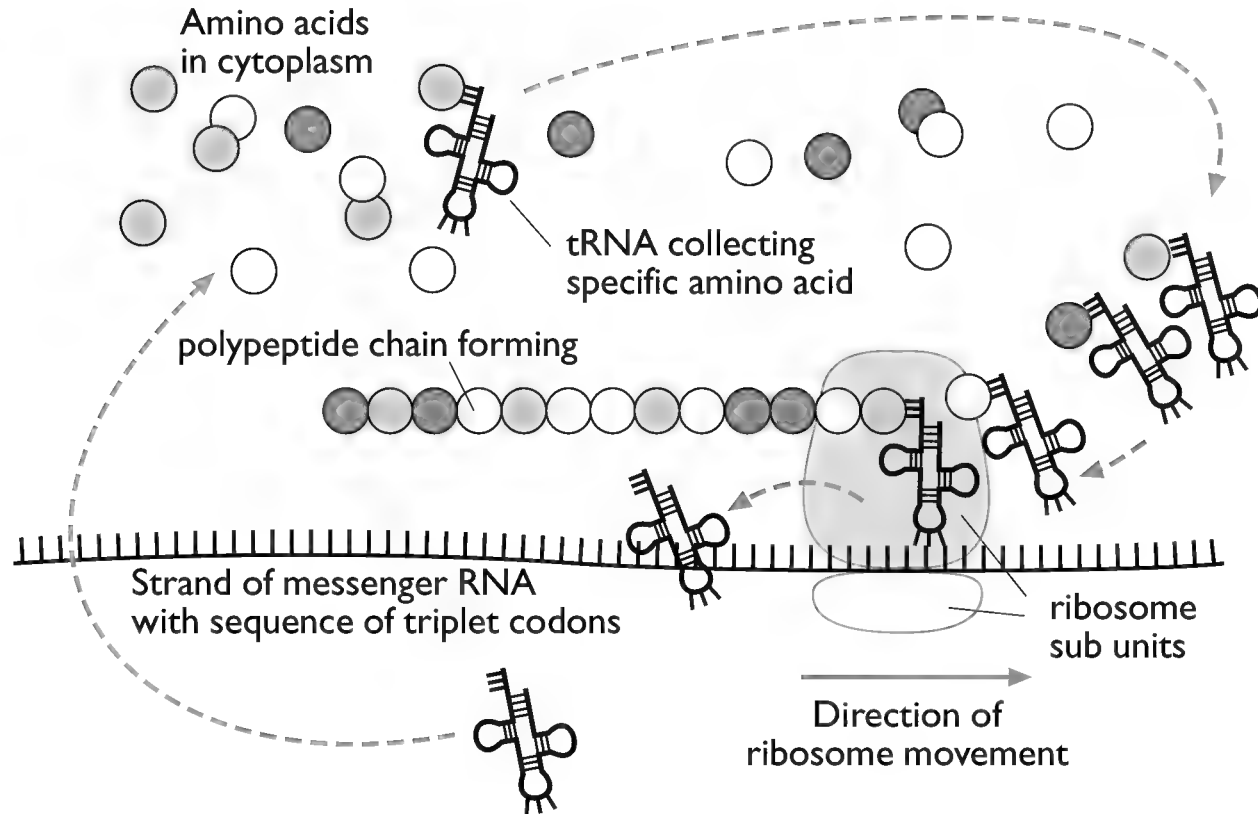
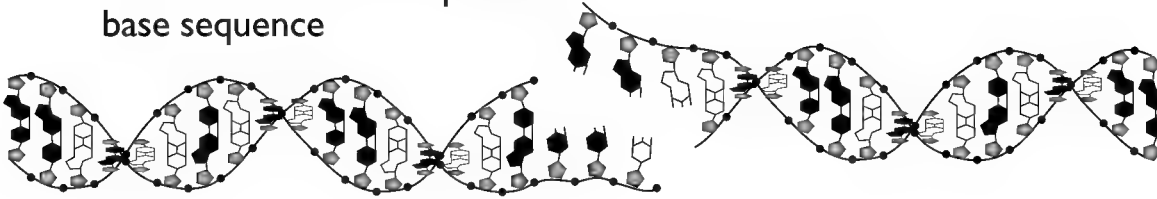


Table of amino acid codons

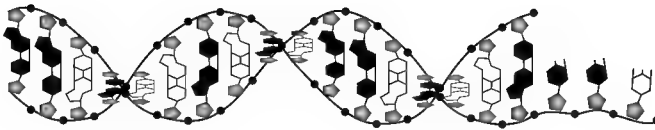
Amino acid	Abbr.	mRNA codons	Essential
Alanine	Ala	GCA, GCC, GCG, GCU	
Arginine	Arg	AGA, AGG, CGA, CGC, CGG, CGU	infants only
Asparagine	Asn	AAC, AAU	
Aspartic acid	Asp	GAC, GAU	
Cysteine	Cys	UGC, UGU	
Glutamic acid	Glu	GAA, GAG	
Glutamine	Gln	CAA, CAG	
Glycine	Gly	GGA, GGC, GGG, GGU	
Histidine	His	CAC, CAU	infants only
Isoleucine	Ile	AUA, AUC, AUU	E
Leucine	Leu	CUA, CUC, CUG, CUU, UUA, UUG	E
Lysine	Lys	AAA, AAG	E
Methionine	Met	AUG (Start codon)	E
Phenylalanine	Phe	UUC, UUU	E
Proline	Pro	CCA, CCC, CCG, CCU	
Serine	Ser	AGC, AGU, UCA, UCC, UCG, UCU	
Threonine	Thr	ACA, ACC, ACG, ACU	E
Tryptophan	Trp	UGG	E
Tyrosine	Tyr	UAC, UAU	
Valine	Val	GUA, GUC, GUG, GUU	E
STOP CODONS		UAA, UAG, UGA	

Action of restriction and ligase enzymes

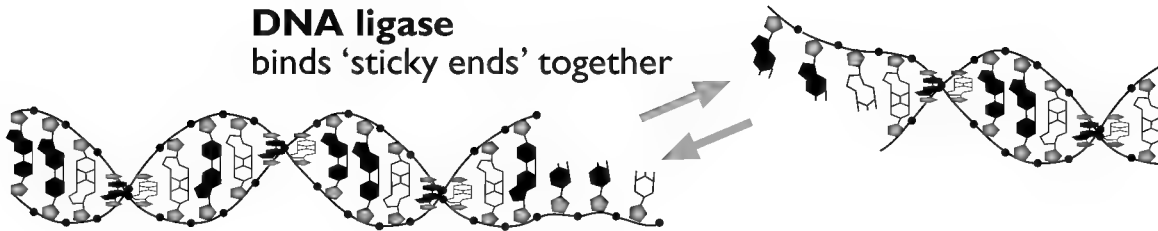
Restriction endonuclease
breaks the chain at a specific
base sequence

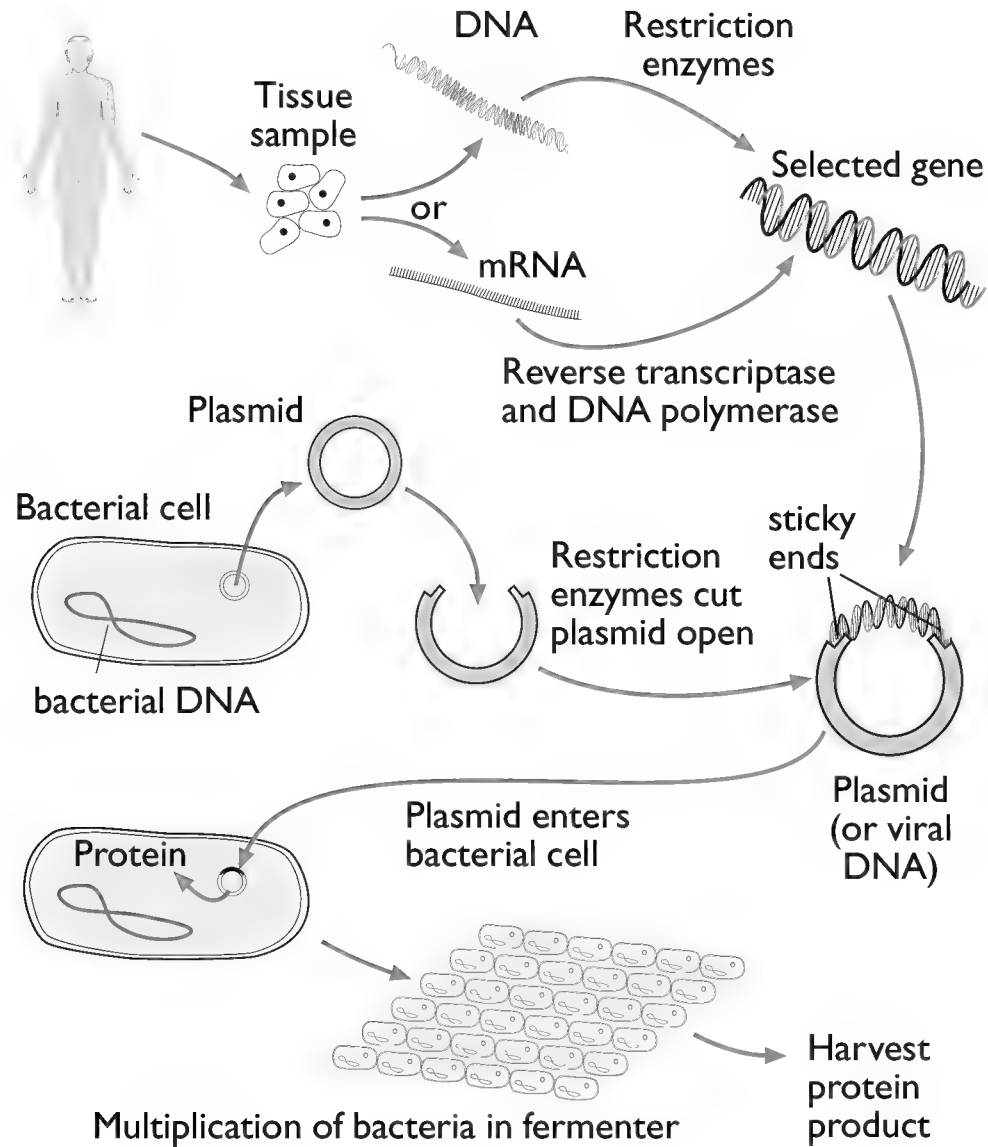


'Sticky end'
results from the staggered cut

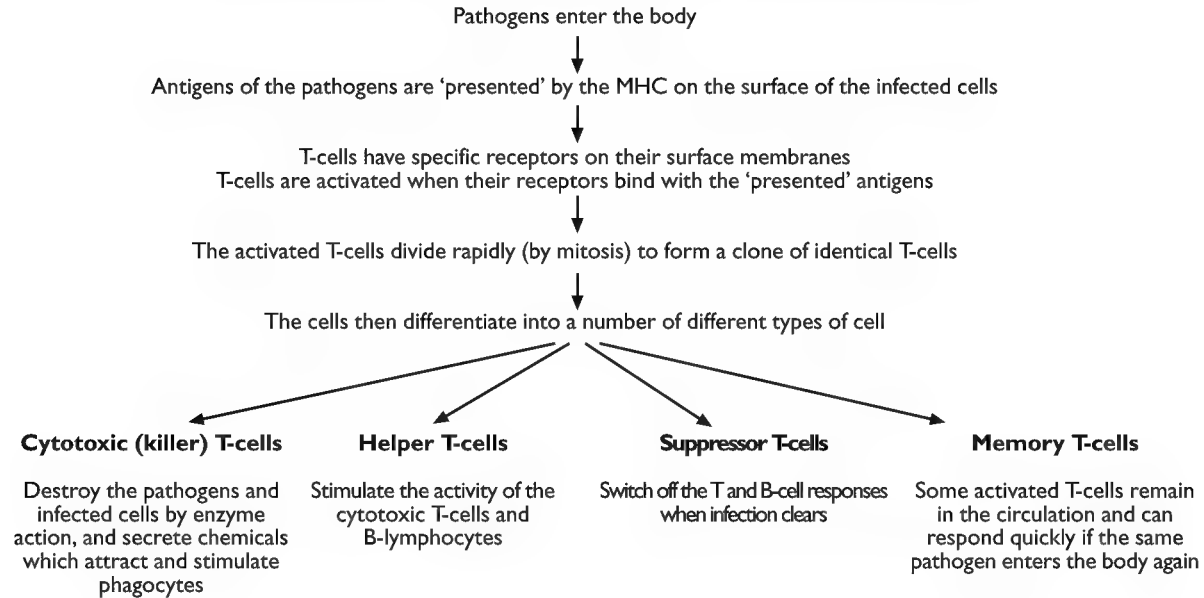


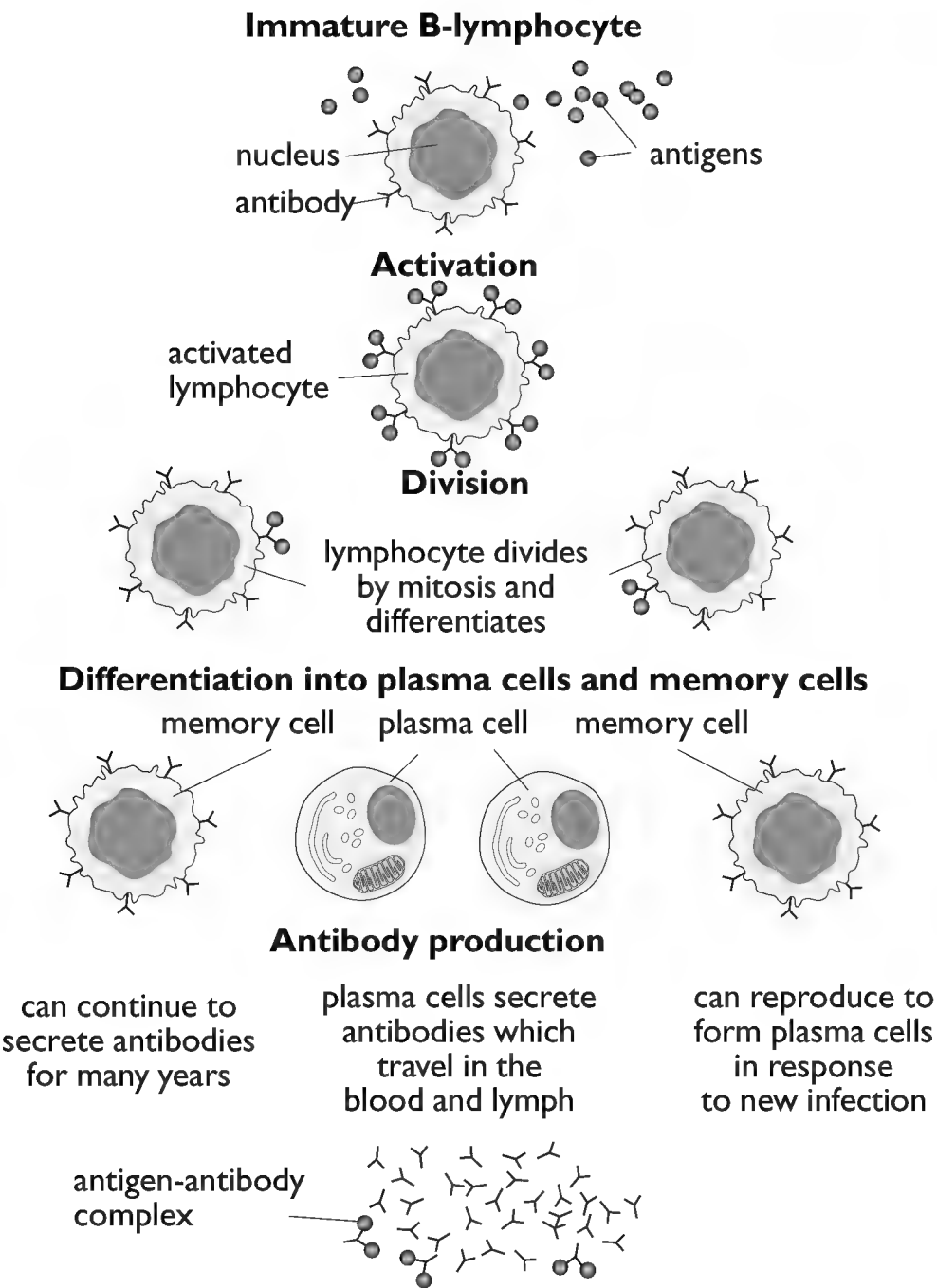
DNA ligase
binds 'sticky ends' together



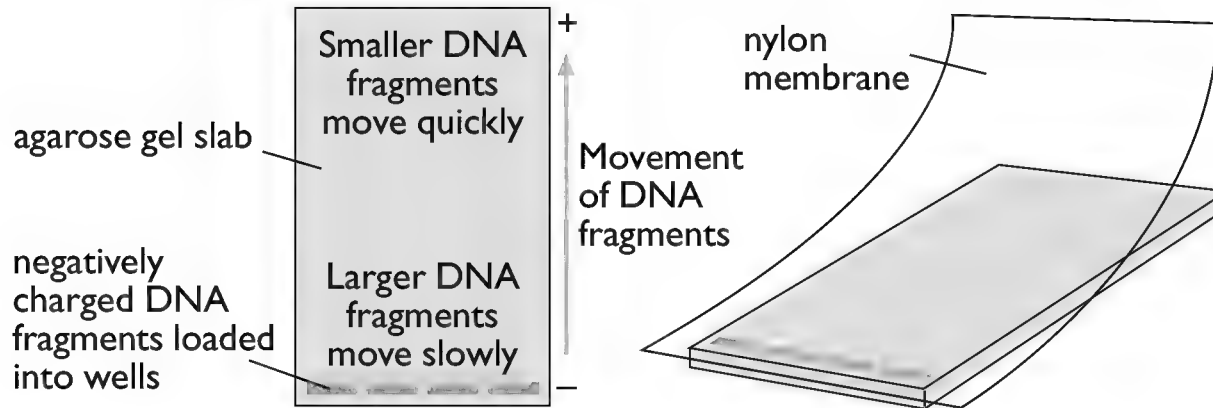


Cellular response to infection - T-lymphocytes (T-cells)

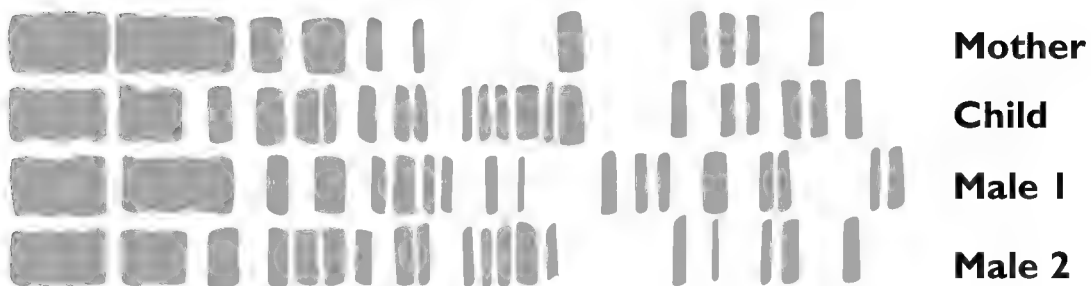
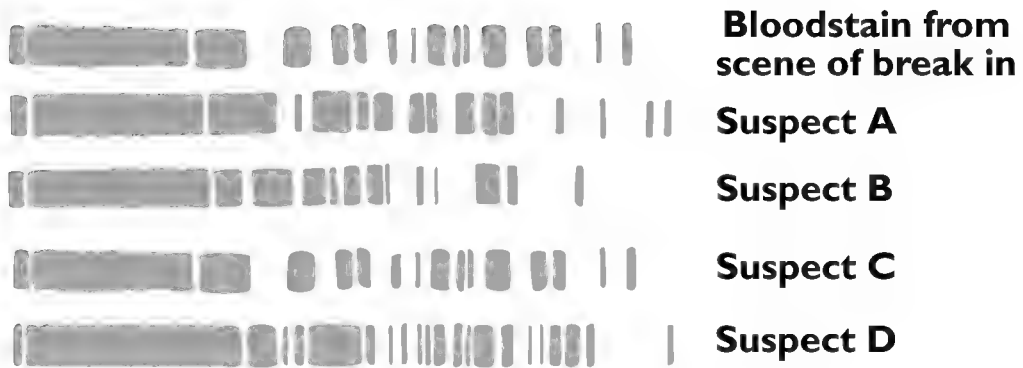




- 1 Extracted DNA e.g. from white blood cells, semen etc.
- 2 DNA cut into pieces by **Restriction Enzymes**
- 3 DNA pieces are separated by **Gel Electrophoresis** this takes 24 hours

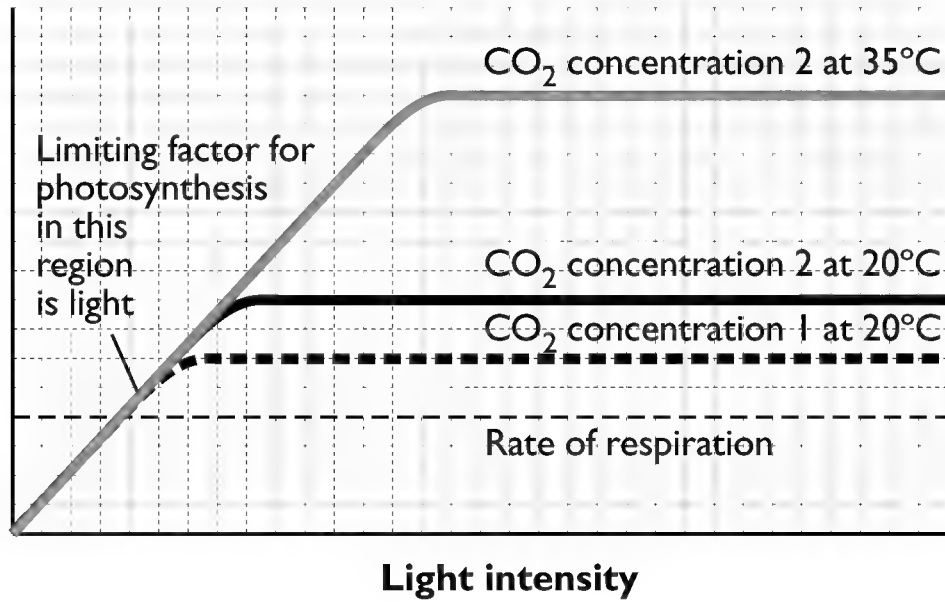


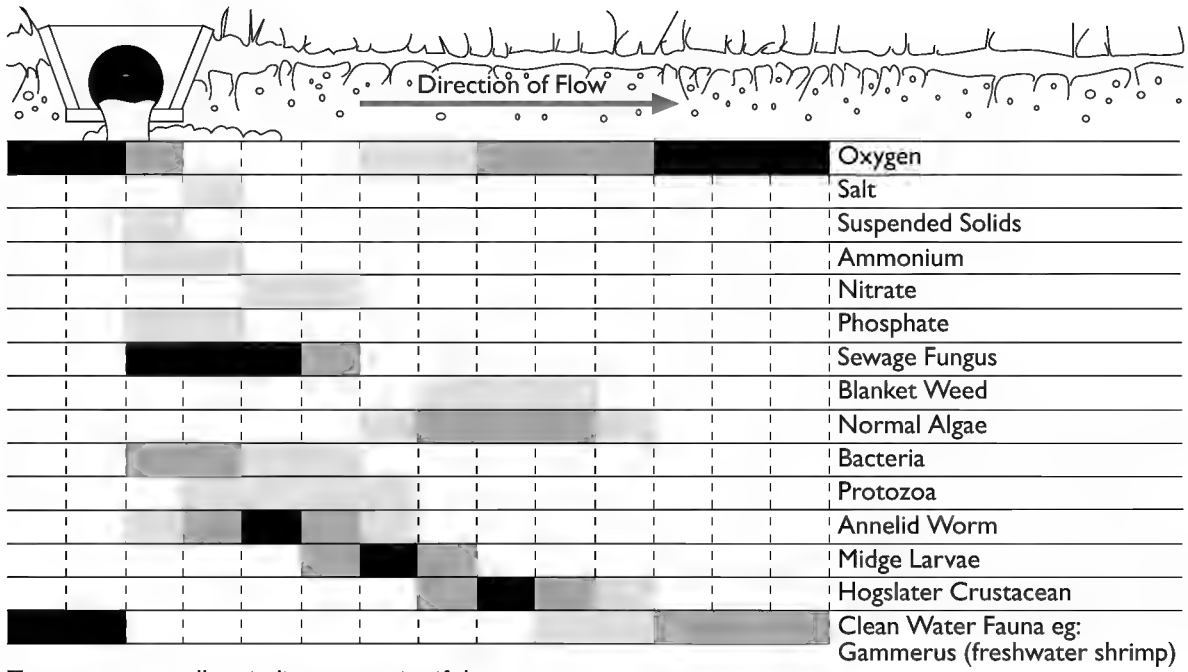
- 4 **Southern Blotting**, the DNA fragments are transferred (blotted) onto a nylon membrane laid on top of the gel slab.
- 5 **Gene Probe Treatment** - the nylon membrane is put into a tank of single strand DNA's (probes) which are complementary to specific core DNA sequences and bind to them. The probes may be radioactive or fluorescent. Excess probes are washed off
- 6 X Ray film is placed on the membrane and the typical bands appear where the presence of **radioactive/fluorescent probes** fog the film.
- 7 The X Ray film is processed to form the **DNA Fingerprint** with its characteristic 'barcode' pattern of dark bands.





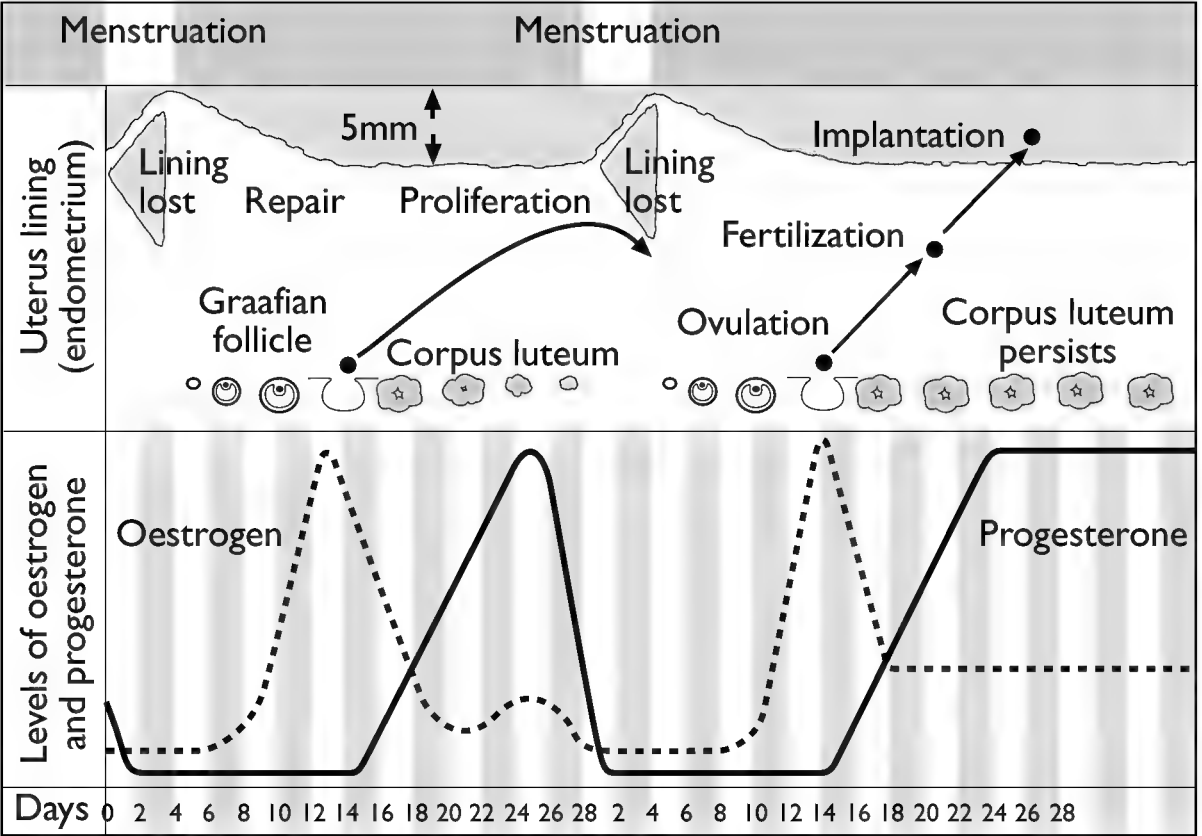
Rate of photosynthesis
(measured as volume of oxygen produced)



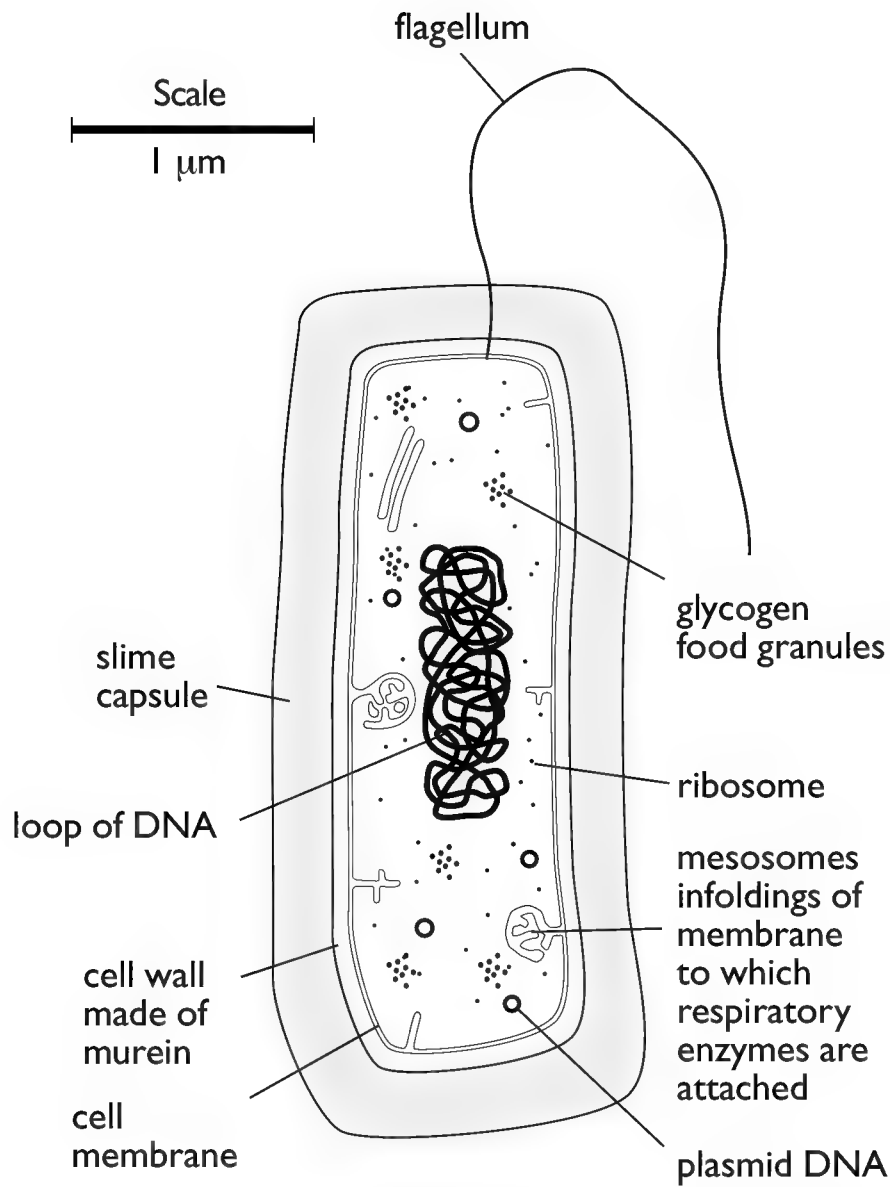


Trout are an excellent indicator species if they survive then the water is fit for almost anything

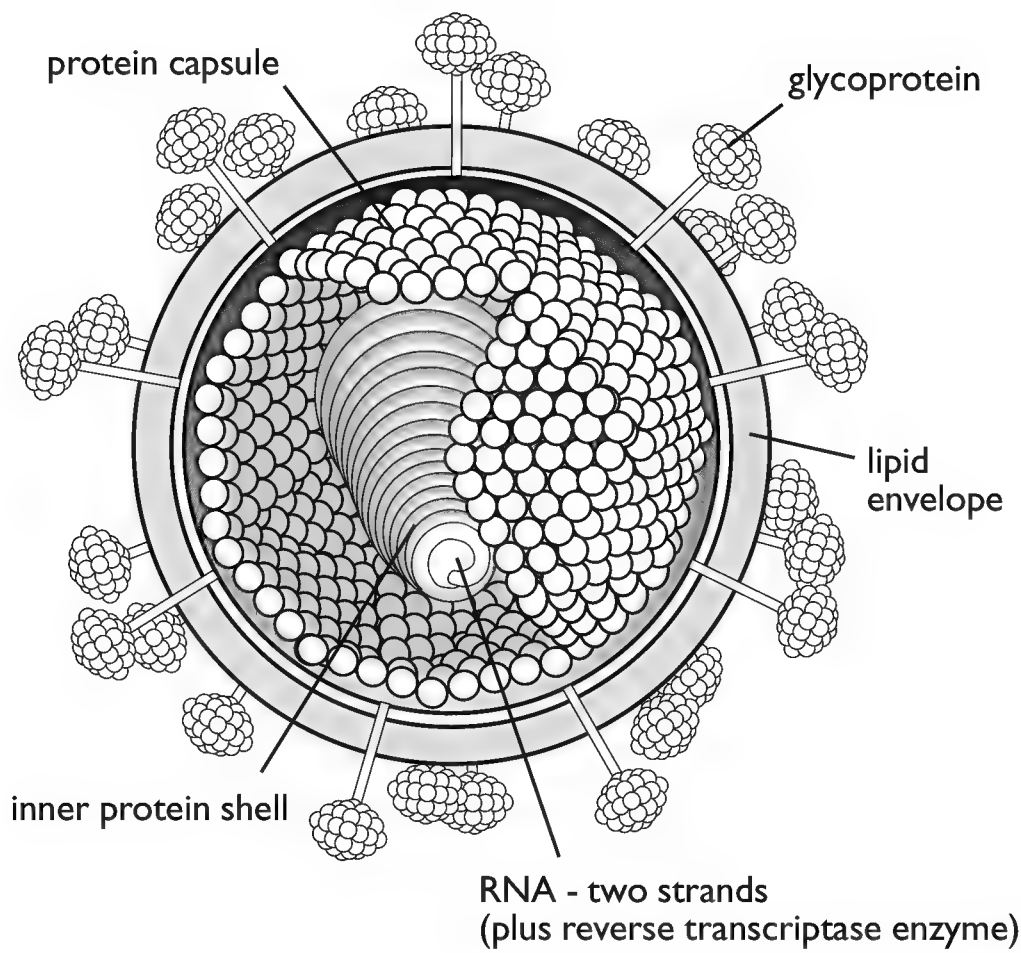
High
Low



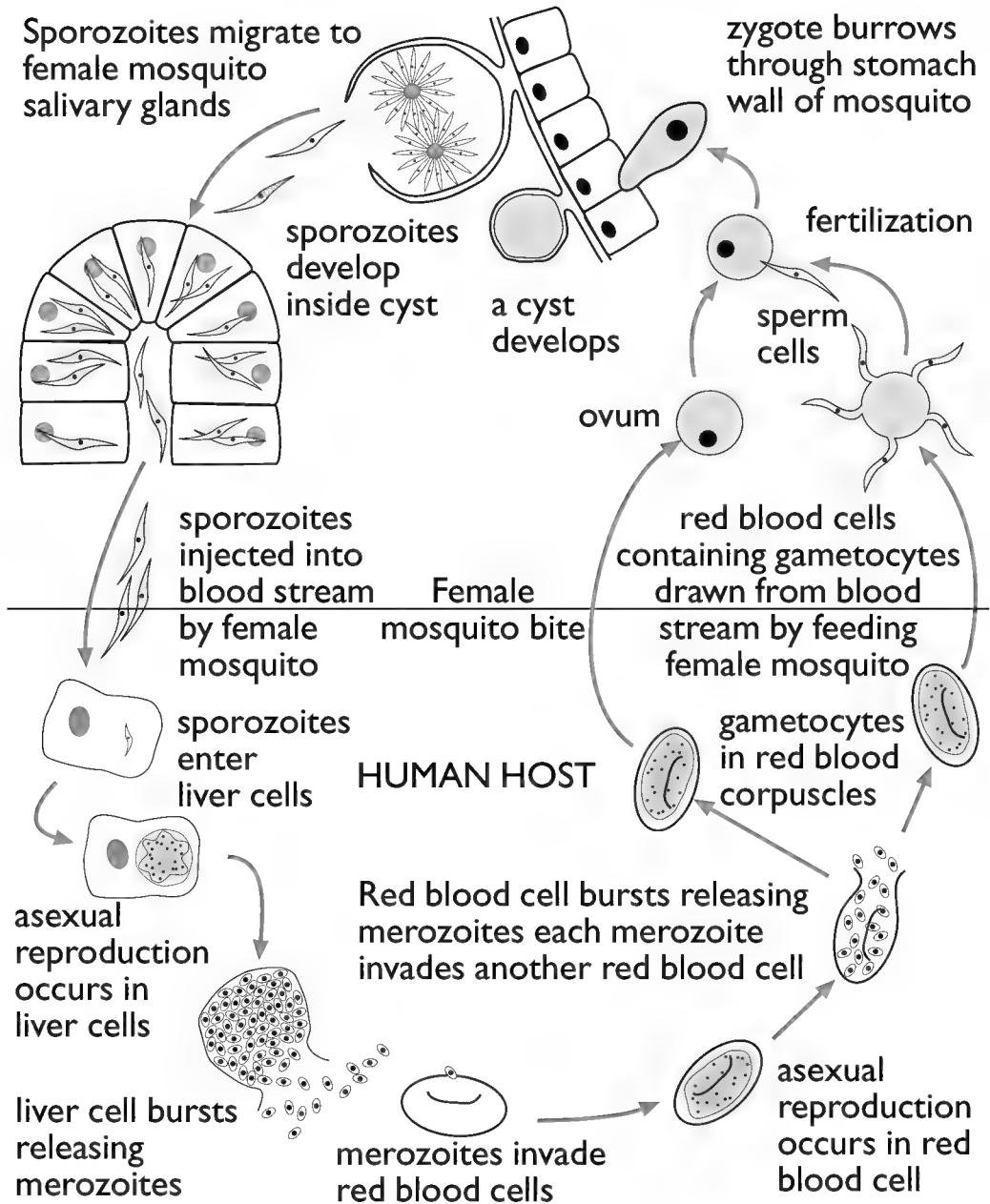
Bacterium

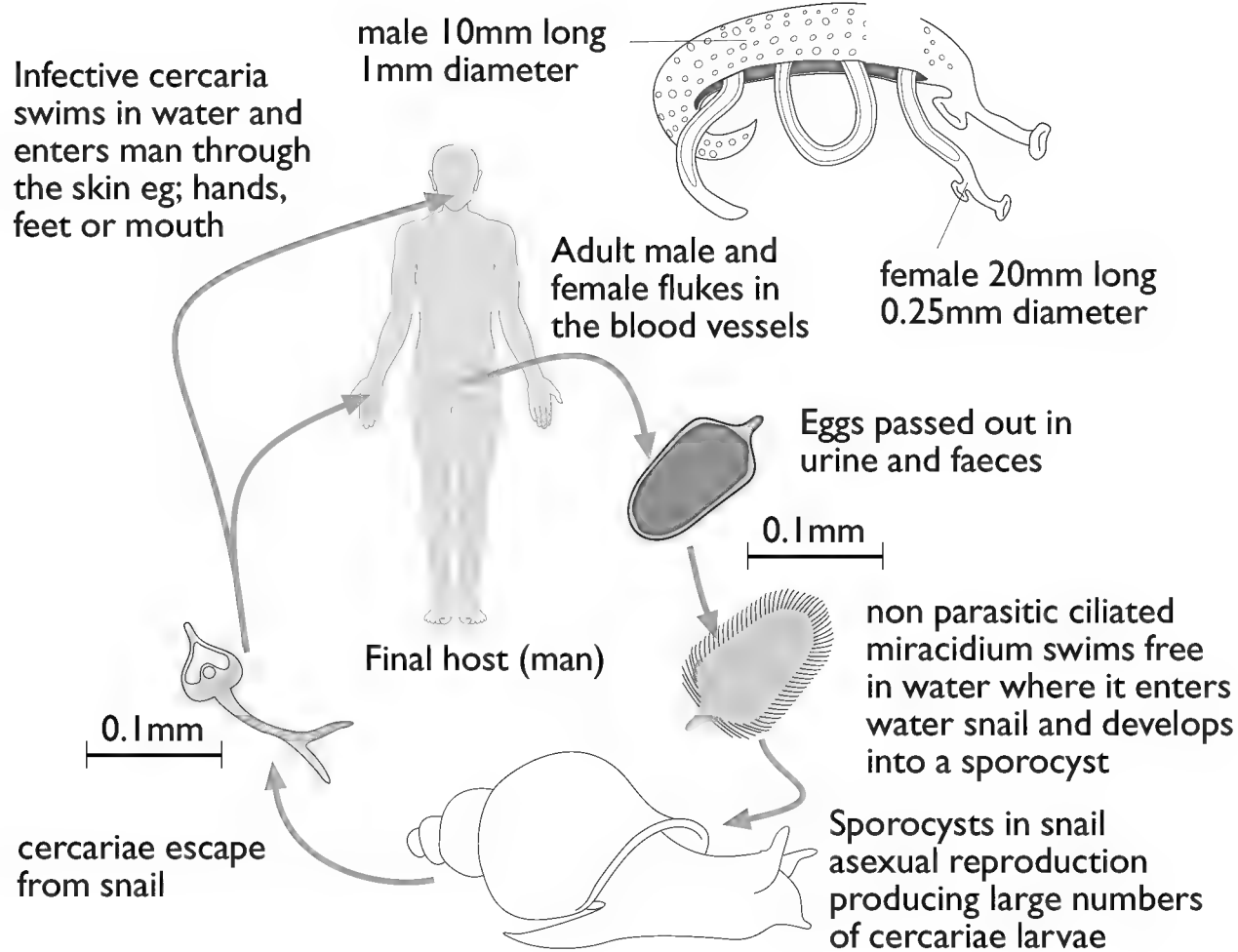


Human Immunodeficiency Virus

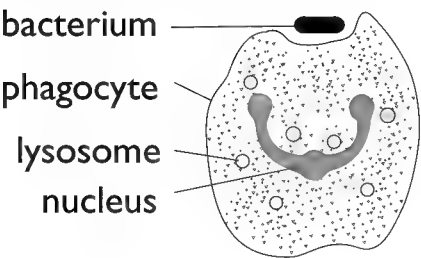


Life cycle of Plasmodium

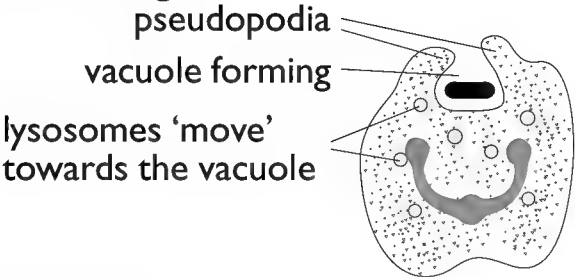




Phagocyte ‘moves’ towards bacterium

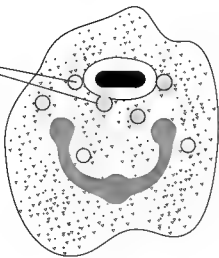


Vacuole forming

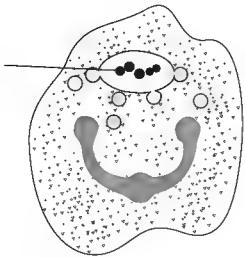


Vacuole formed

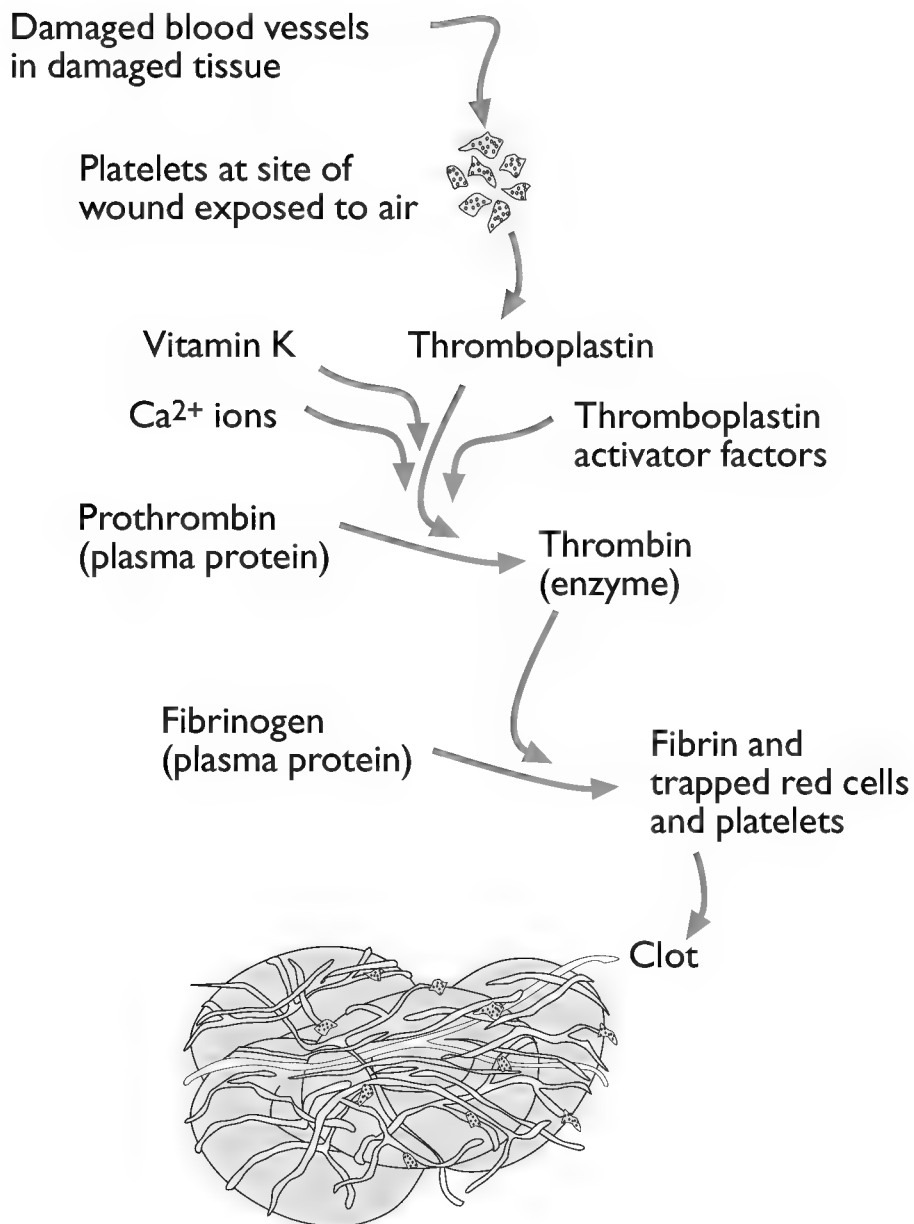
lysosomes collect around the vacuole fuse with it releasing digestive enzymes

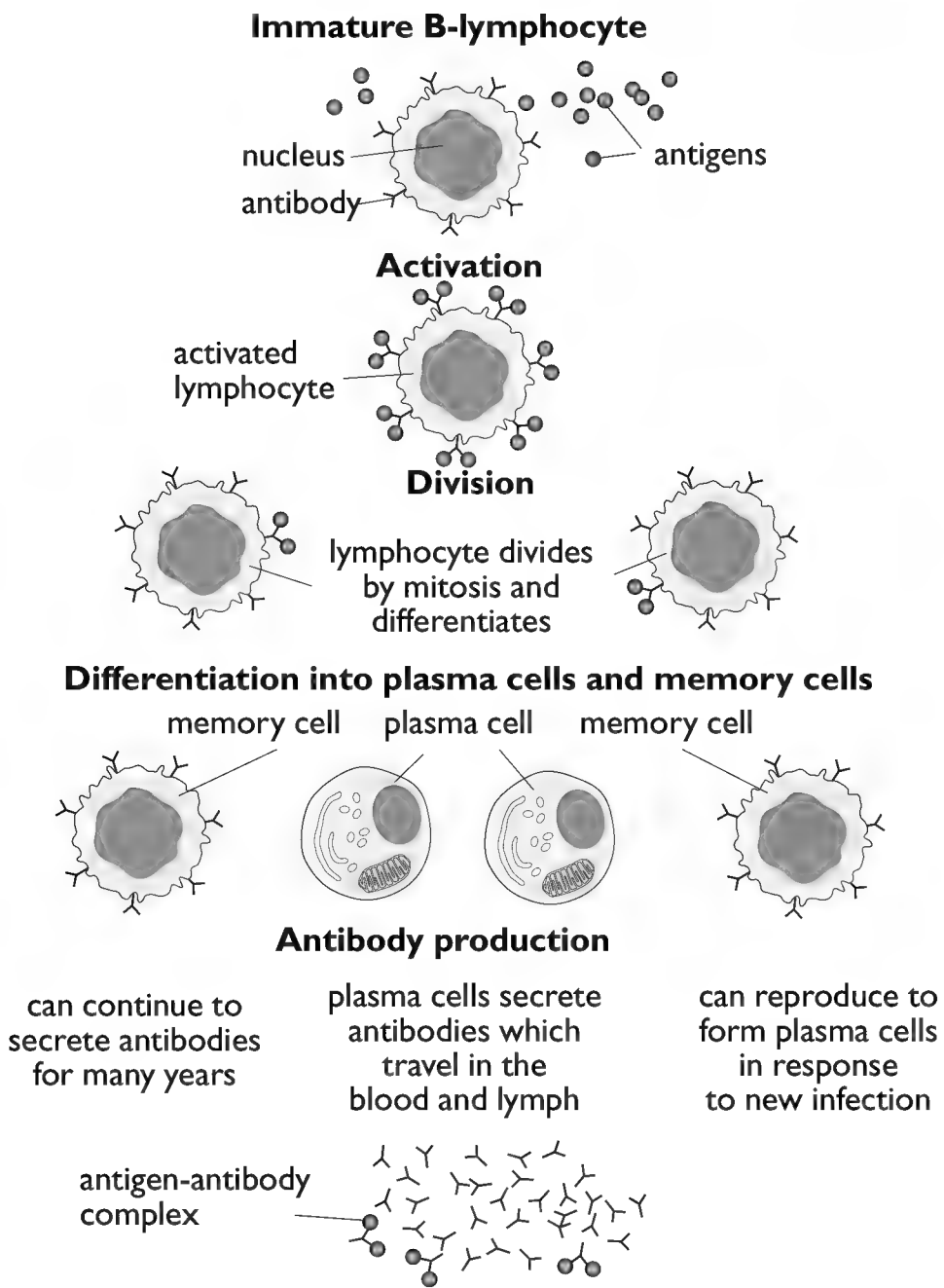


bacterium digested and products absorbed

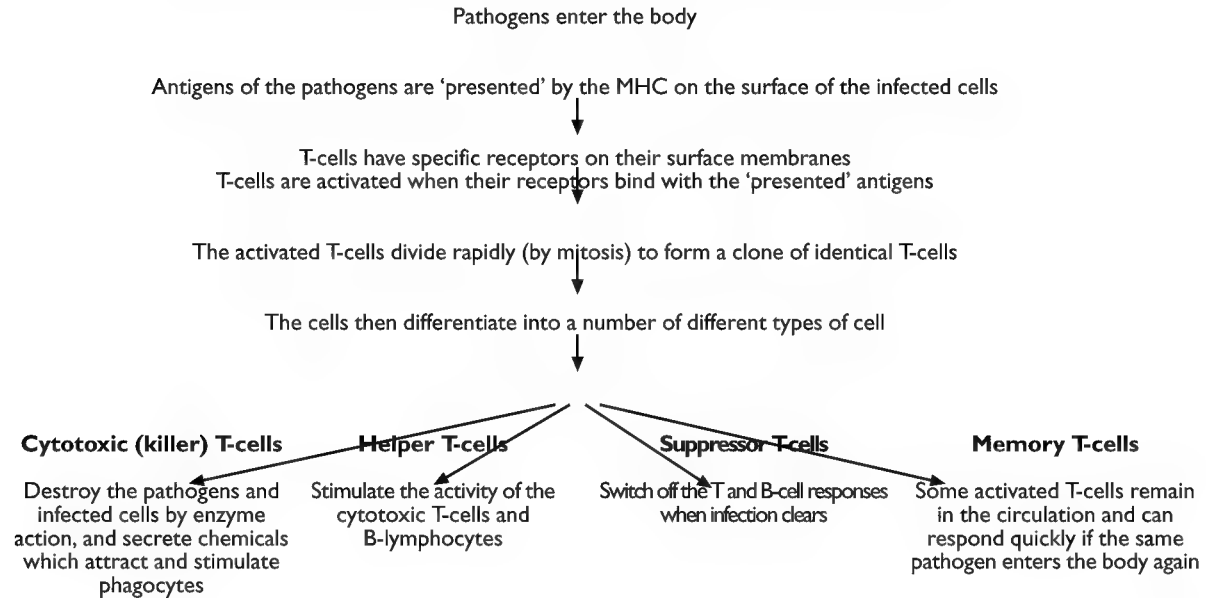


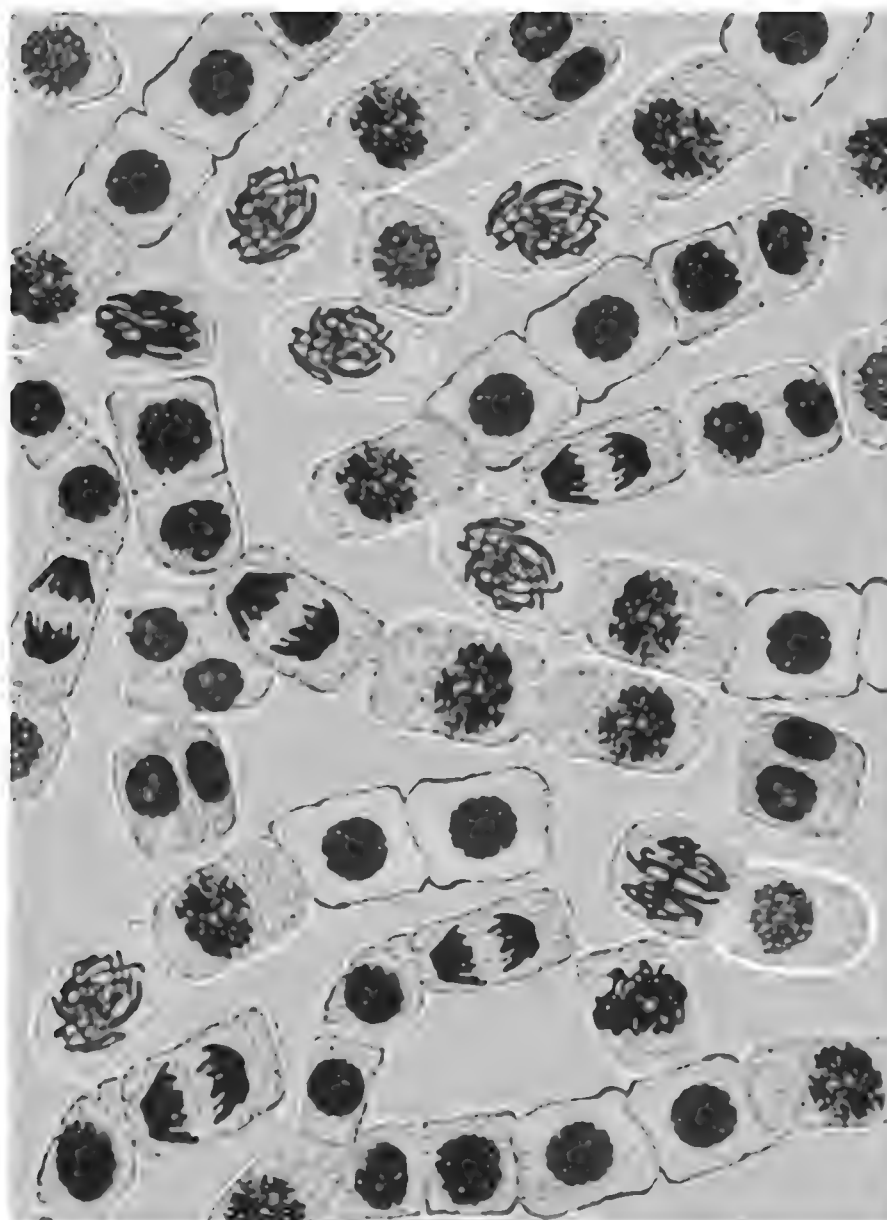
Blood clotting



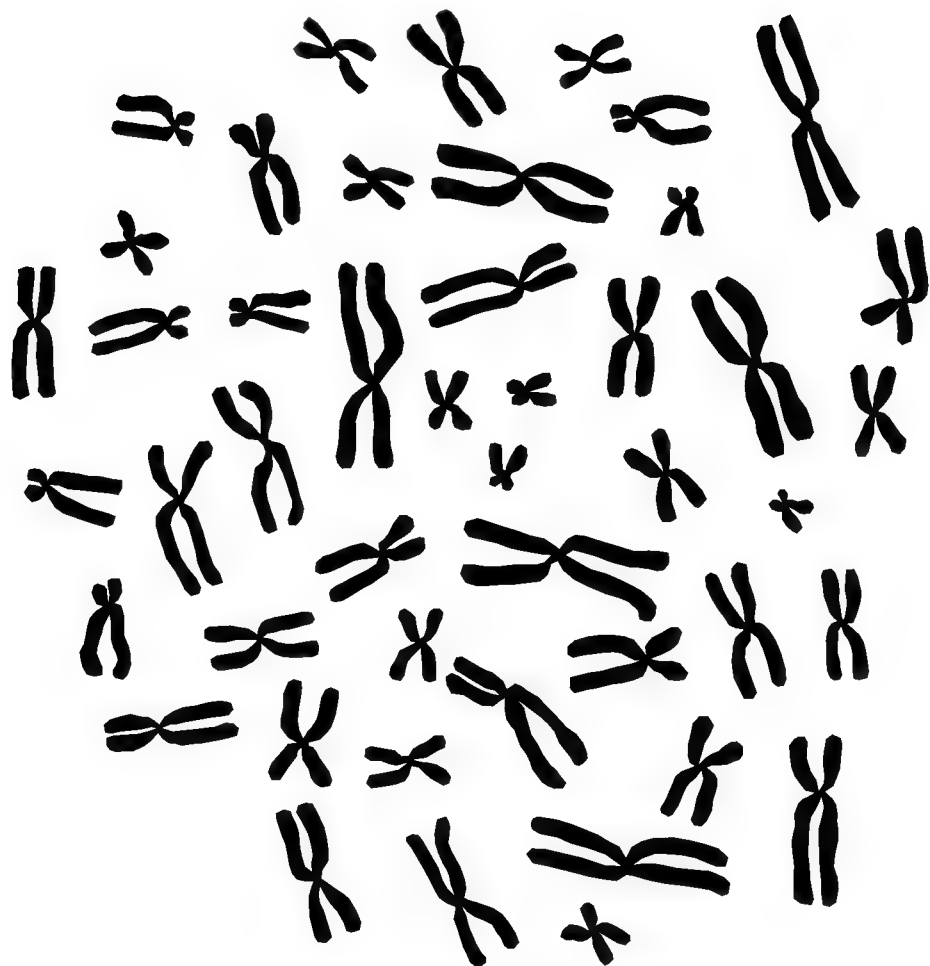


Cellular response to infection - T-lymphocytes (T-cells)



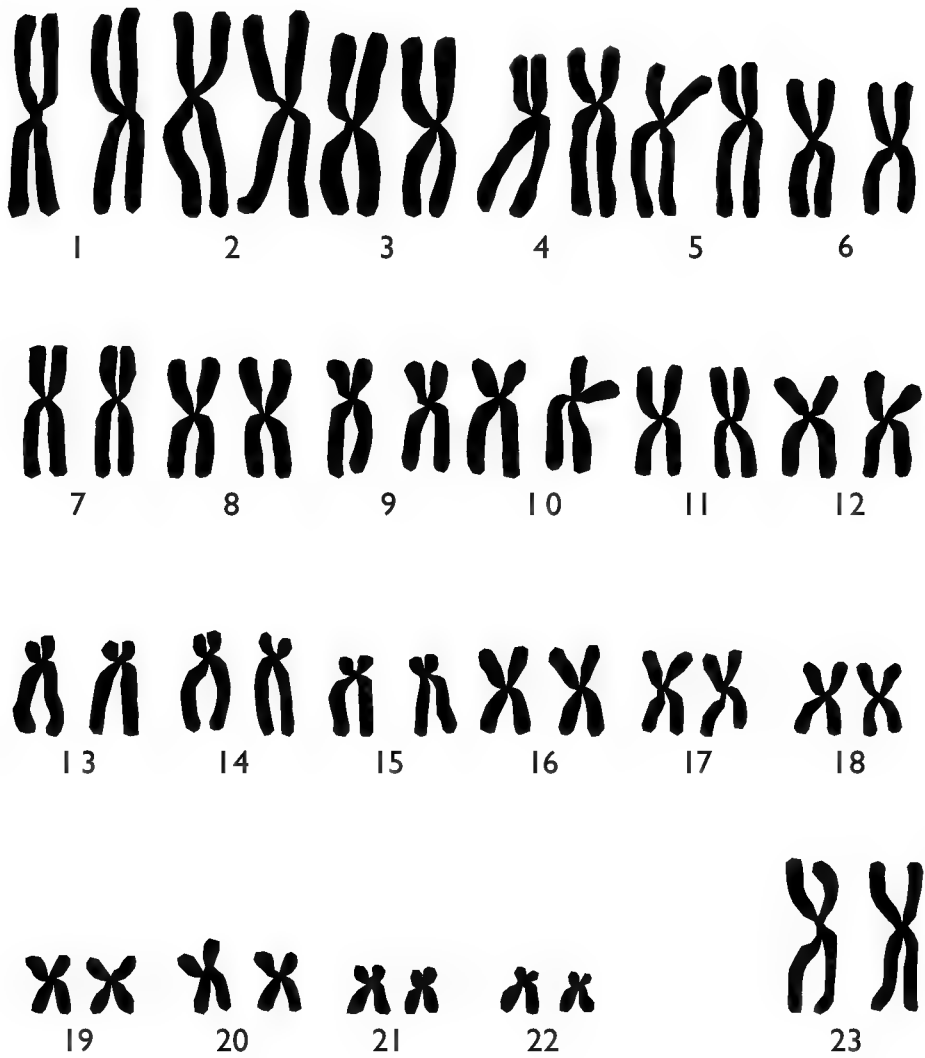


Normal human karyotype



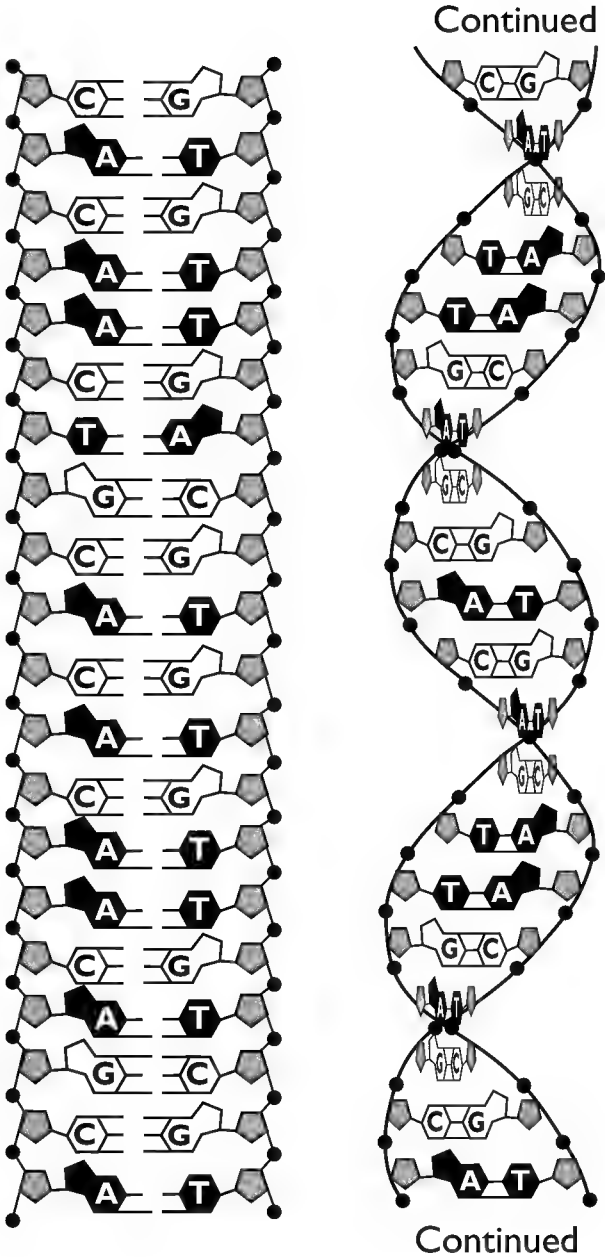
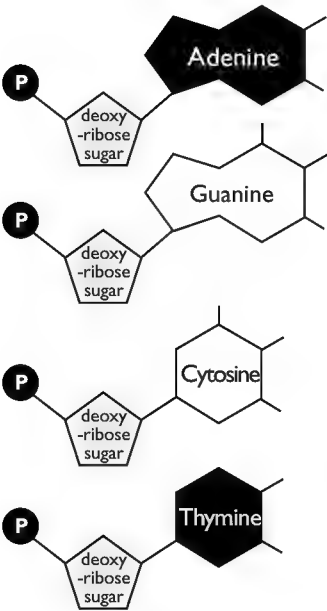
Human karyotype analysed into homologous pairs

Normal Human Female

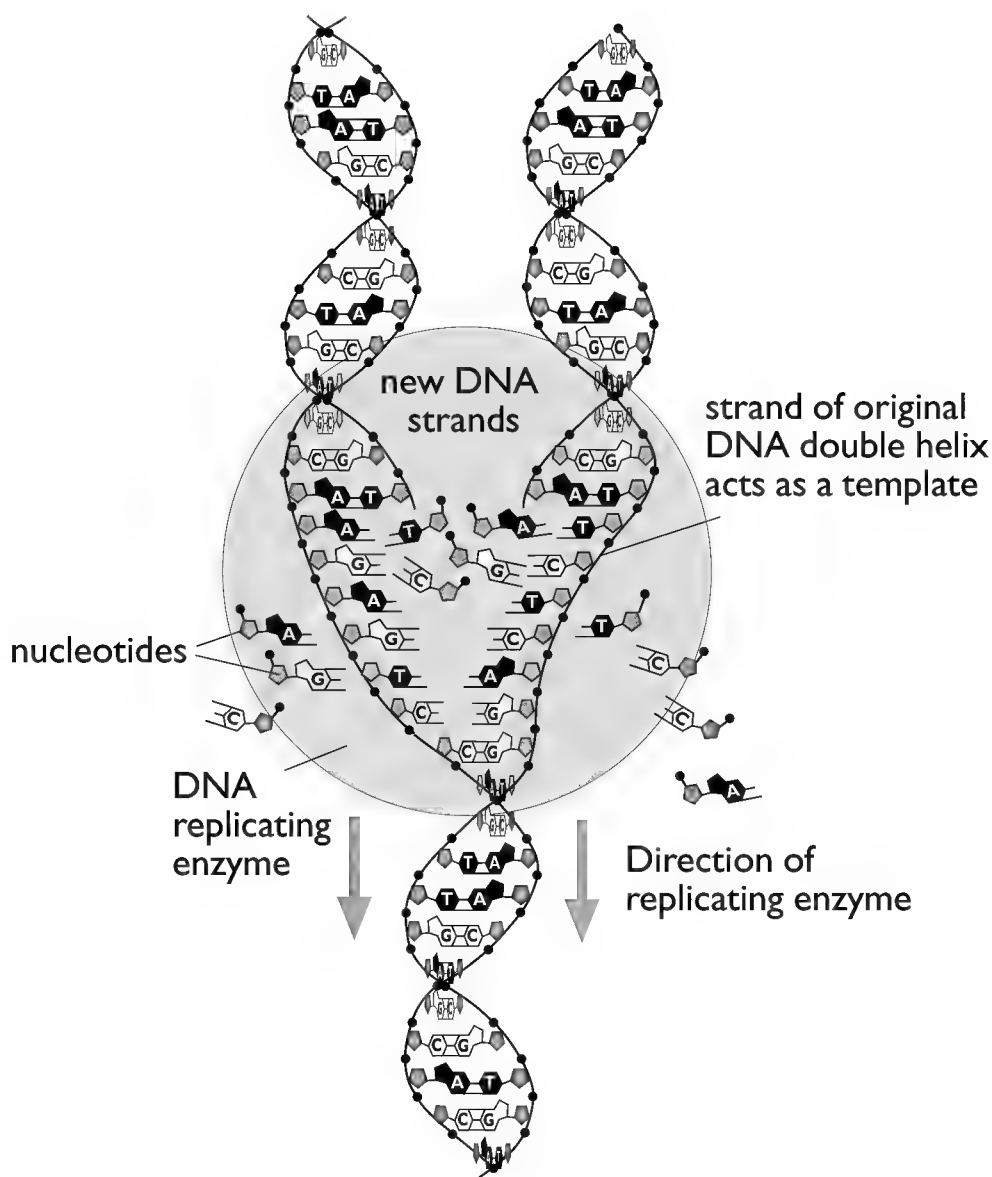


Structure of DNA

The Nucleotides of DNA

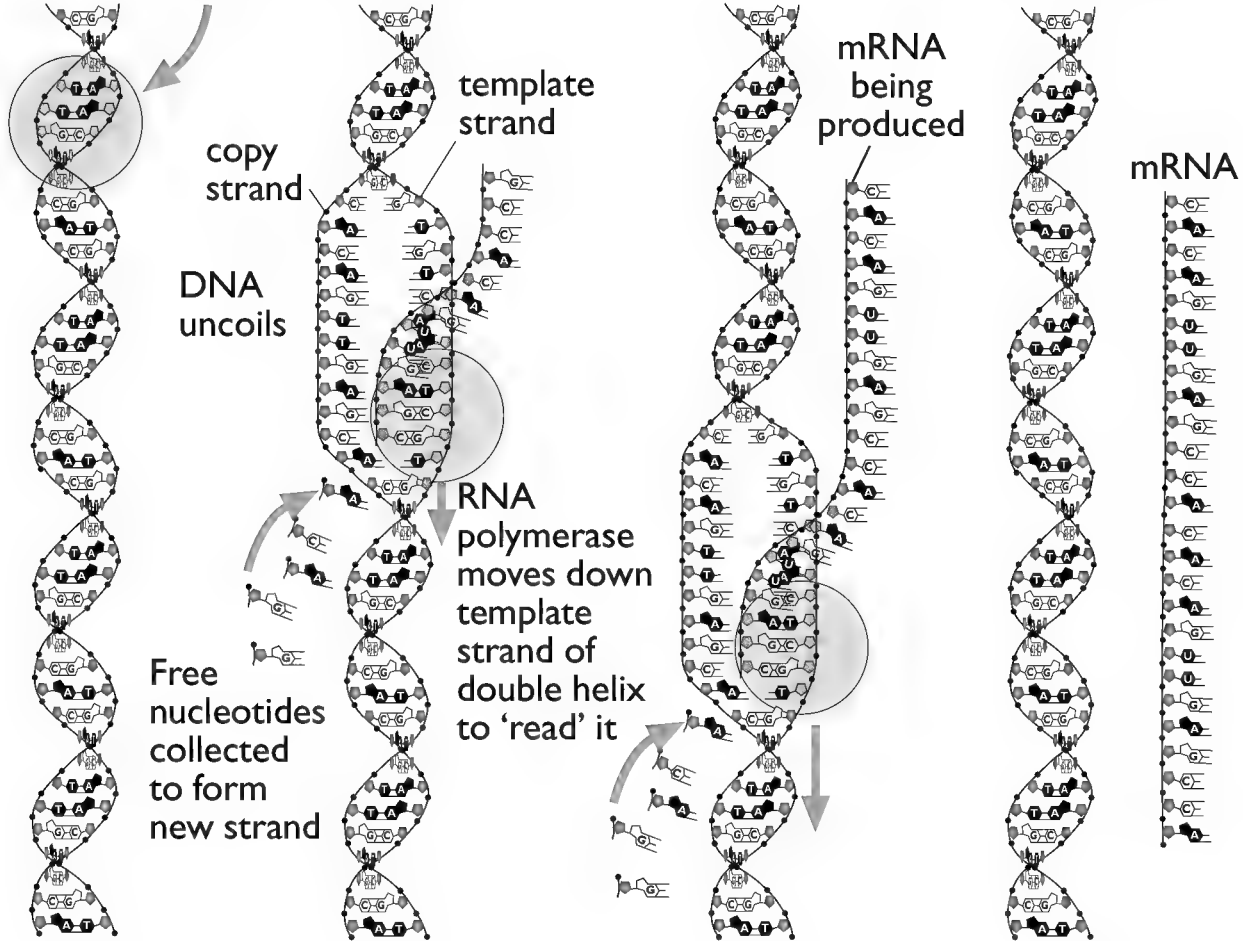


Replication of DNA



RNA polymerase attaches to DNA strand at specific codon site

DNA double helix rewinds to original



Translation of mRNA code to build a polypeptide

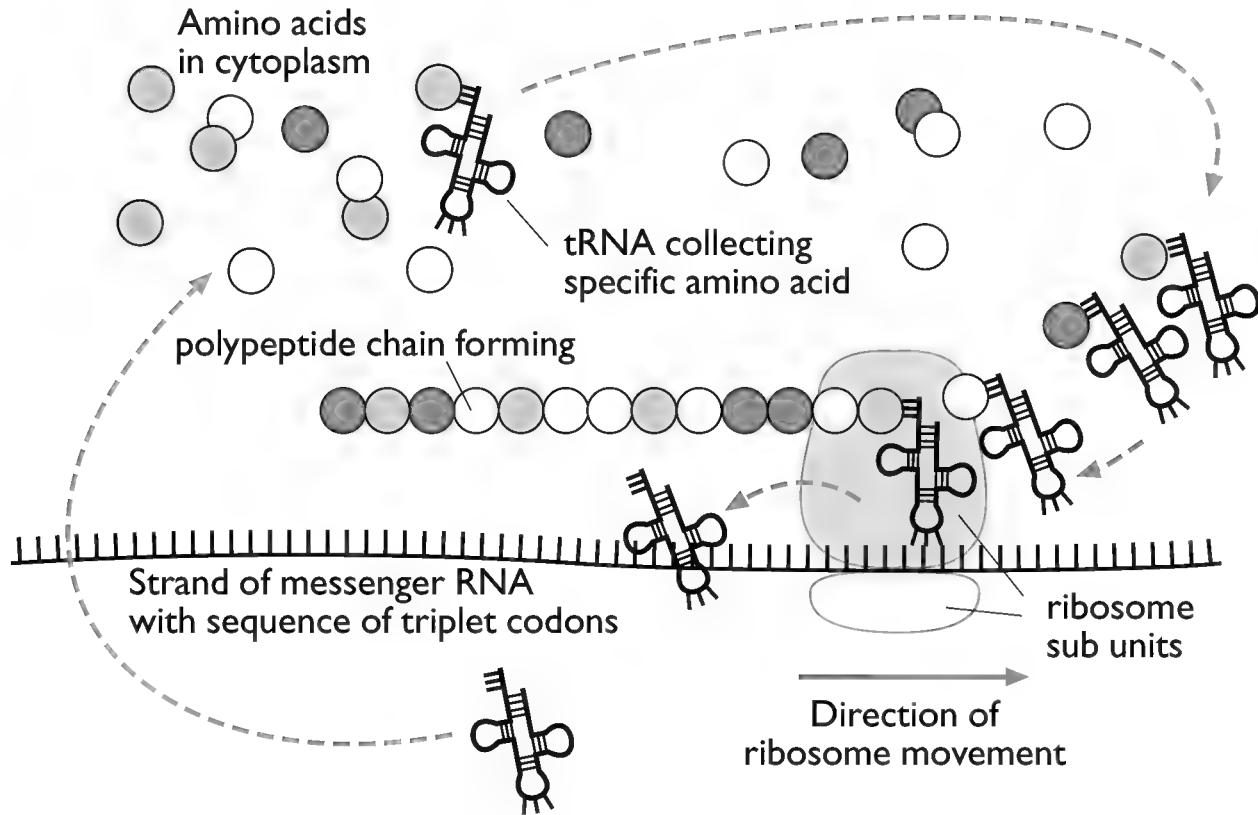
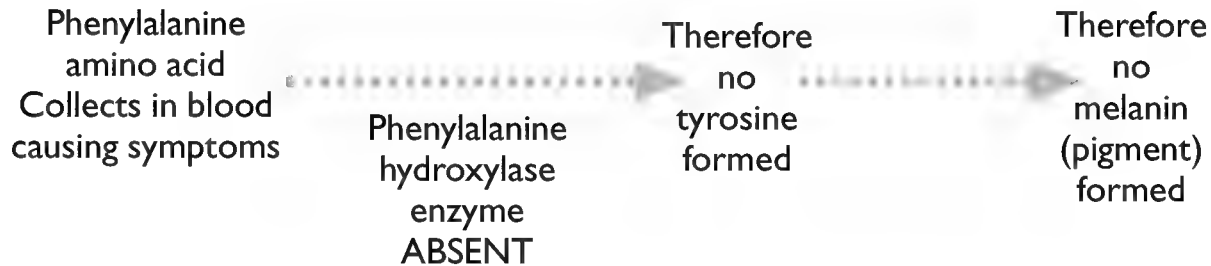
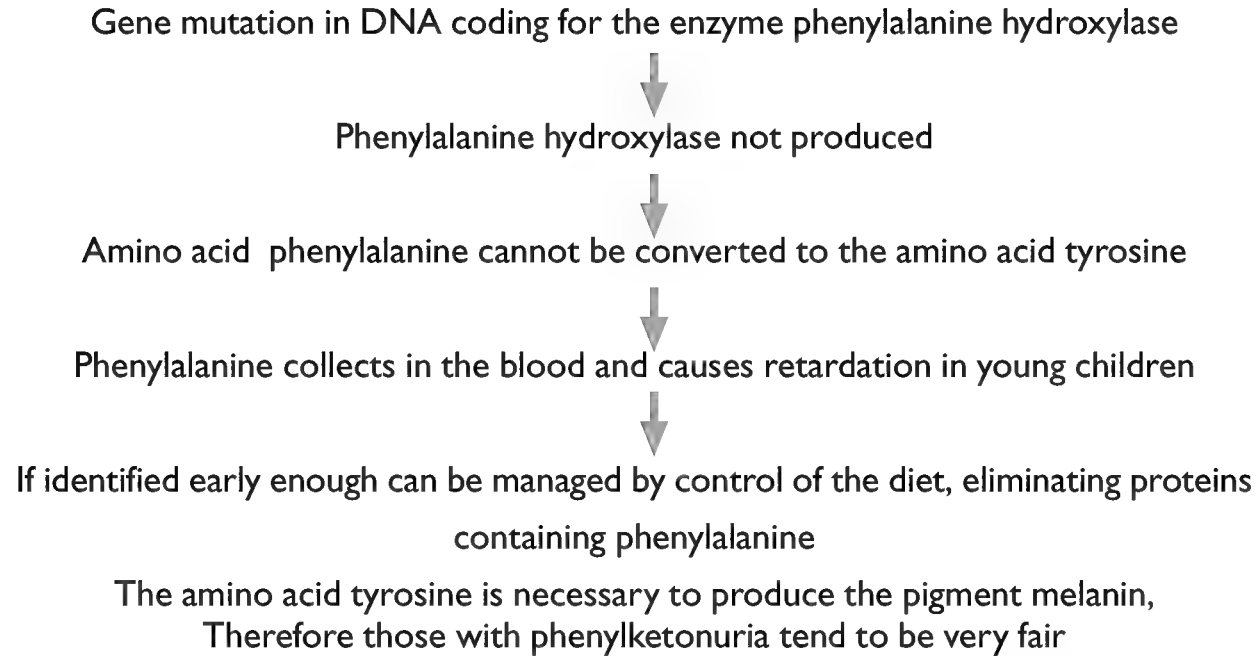


Table of Amino Acid Codons

Amino acid	Abbr.	mRNA codons	Essential
Alanine	Ala	GCA, GCC, GCG, GCU	
Arginine	Arg	AGA, AGG, CGA, CGC, CGG, CGU	infants only
Asparagine	Asn	AAC, AAU	
Aspartic acid	Asp	GAC, GAU	
Cysteine	Cys	UGC, UGU	
Glutamic acid	Glu	GAA, GAG	
Glutamine	Gln	CAA, CAG	
Glycine	Gly	GGA, GGC, GGG, GGU	
Histidine	His	CAC, CAU	infants only
Isoleucine	Ile	AUA, AUC, AUU	E
Leucine	Leu	CUA, CUC, CUG, CUU, UUA, UUG	E
Lysine	Lys	AAA, AAG	E
Methionine	Met	AUG (Start codon)	E
Phenylalanine	Phe	UUC, UUU	E
Proline	Pro	CCA, CCC, CCG, CCU	
Serine	Ser	AGC, AGU, UCA, UCC, UCG, UCU	
Threonine	Thr	ACA, ACC, ACG, ACU	E
Tryptophan	Trp	UGG	E
Tyrosine	Tyr	UAC, UAU	
Valine	Val	GUA, GUC, GUG, GUU	E
STOP CODONS		UAA, UAG, UGA	



Cystic Fibrosis

Mutation causes the deletion of 3 bases in DNA
so that one amino acid (phenylalanine)
is not coded for in the CFTR protein
(Cystic fibrosis transmembrane regulator)



Faulty CFTR protein cannot control the opening of
chloride channels in the cell membrane



Results in production of thick sticky mucus,
especially in lungs, pancreas and liver
Organs cannot function normally
and infection becomes more likely

Treatment by Gene Therapy

CFTR gene identified on chromosome 7 in 1989

The normal gene for CFTR protein is inserted into
a bacterial plasmid (DNA).

This is enclosed in a liposome (hollow lipid container)

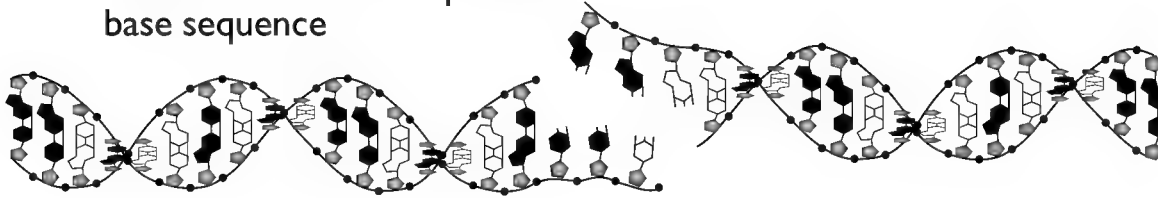
The liposomes are introduced into the body
of the sufferer using a nasal spray

The liposomes reach the lung epithelial cells and
hopefully some of the normal CFTR genes are
incorporated into the cells' DNA.

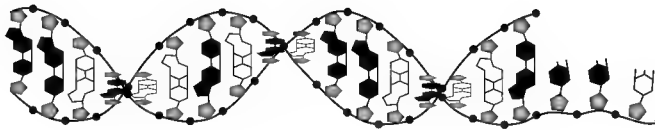
(The normal gene could also be introduced using a 'safe' virus)

Action of restriction and ligase enzymes

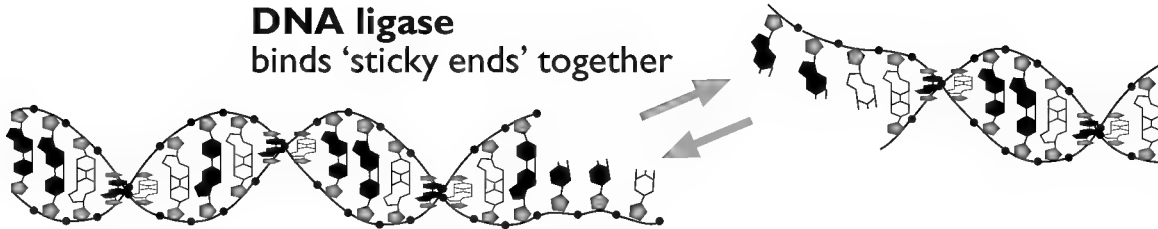
Restriction endonuclease
breaks the chain at a specific
base sequence

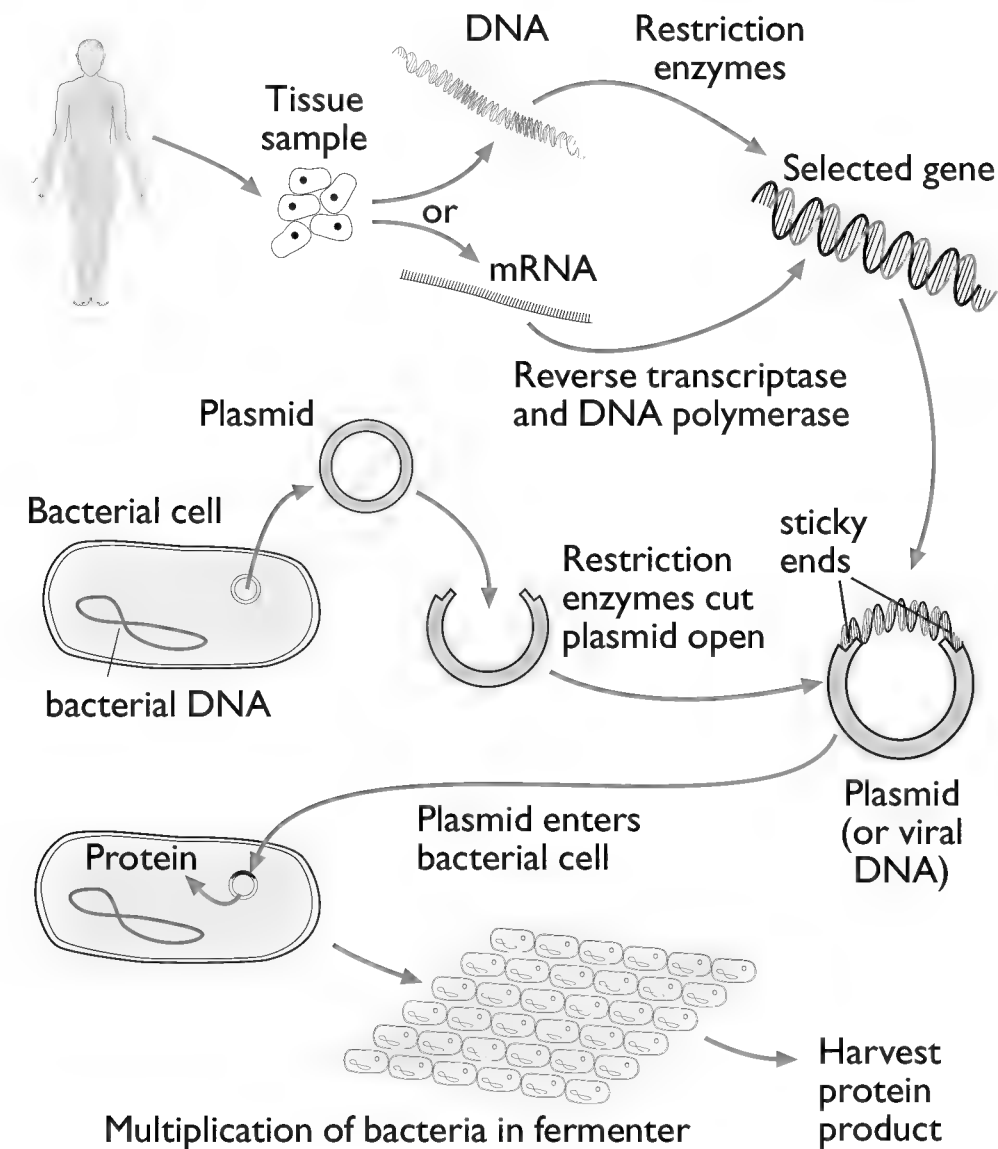


'Sticky end'
results from the staggered cut



DNA ligase
binds 'sticky ends' together

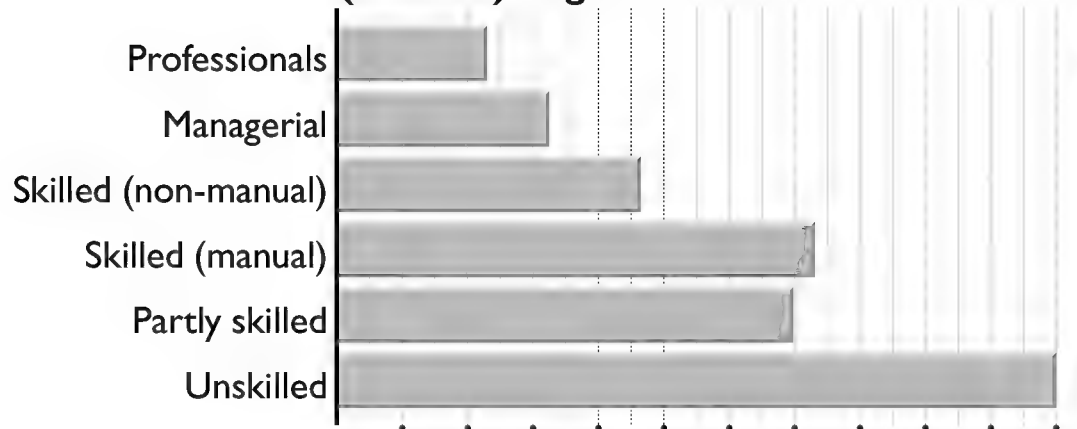




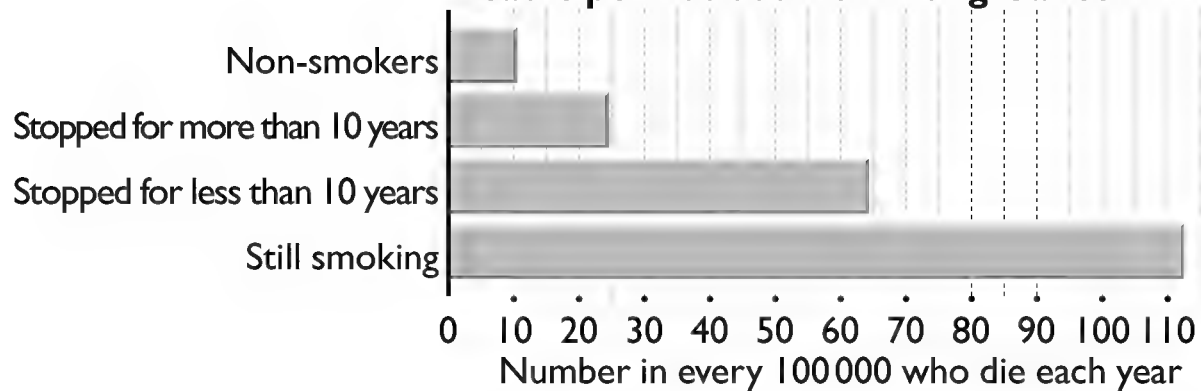
Risk factors in heart disease chart

Age	Sex	Weight	% Animal Fat in Diet	
10-20	Female under 40	More than 2kg below ideal	No animal fat	
21-30	Female under 40-50	Within 2kg of ideal	10% animal fat	
31-40	Female under over 50	15kg over ideal	20% animal fat	
41-50	Male	20kg over ideal	30% animal fat	
51-60	Stocky male	25kg over ideal	40% animal fat	
61 & over	Bald stocky male	30kg over ideal	50% animal fat	Total 1
+ + + =				
Exercise	Tobacco smoking	History of heart disease	Blood pressure	
Hard manual job & exercise	Non smoker	None	Upper reading 100	
Manual job & medium exercise	Cigar or pipe smoker	1 relative over 60	Upper reading 120	
Office job & hard exercise	10 cigs. aday or less	2 relatives over 60	Upper reading 140	
Office job & medium exercise	20 cigarettes aday	1 relative under 60	Upper reading 160	
Office job & light exercise	30 cigarettes aday	2 relatives under 60	Upper reading 180	
No exercise	40 cigarettes aday	3 relatives under 60	Upper reading 200 or more	Total 2
+ + + =				
The higher the total the higher the risk of CHD			Grand Total = _____	

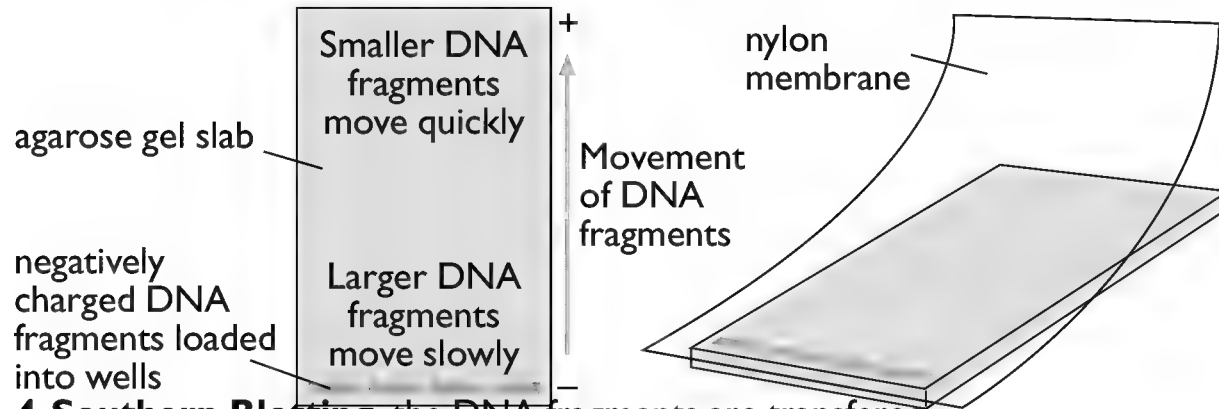
Deaths per 100 000 from Lung Cancer (1991-93) England & Wales



Deaths per 100 000 from Lung Cancer

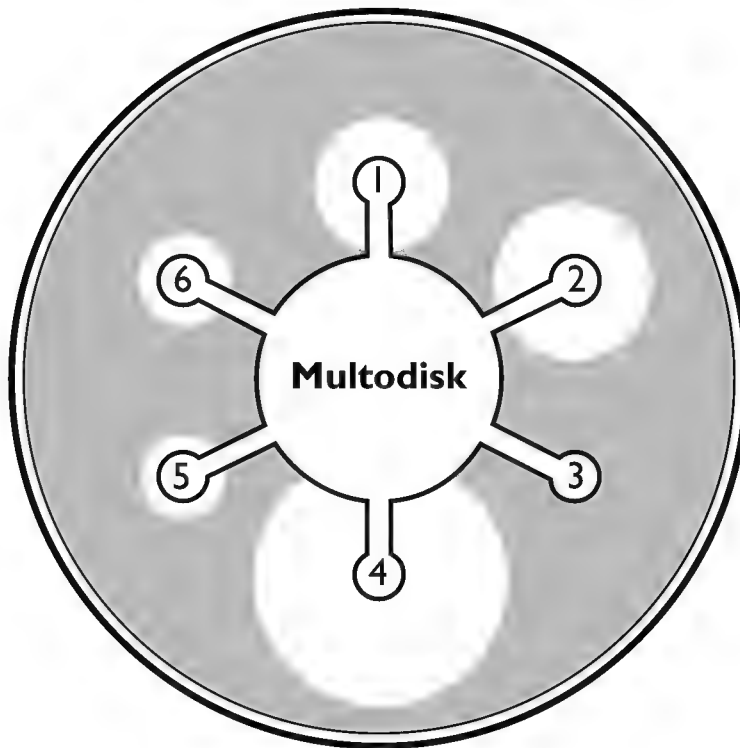


- 1 Extracted DNA e.g. from white blood cells, semen etc.
- 2 DNA cut into pieces by **Restriction Enzymes**
- 3 DNA pieces are separated by **Gel Electrophoresis** this takes 24 hours



- 4 **Southern Blotting**, the DNA fragments are transferred (blotted) onto a nylon membrane laid on top of the gel slab.
- 5 **Gene Probe Treatment** - the nylon membrane is put into a tank of single strand DNA's (probes) which are complementary to specific core DNA sequences and bind to them. The probes may be radioactive or fluorescent. Excess probes are washed off
- 6 X Ray film is placed on the membrane and the typical bands appear where the presence of **radioactive/fluorescent probes** fog the film.
- 7 The X Ray film is processed to form the **DNA Fingerprint** with its characteristic 'barcode' pattern of dark bands.

Diagram to show action of specific antibiotics on a bacterial culture



- 1 Tetracycline
- 2 Chloraphenol
- 3 Erythromycin
- 4 Sulphafurazole
- 5 Penicillin G
- 6 Streptomycin

Biology



- 1 - Molecules, Cells & Systems**
- 2 - Making Use of Biology**
- 3 - Pathogens & Disease**

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Practical Guide



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Practical Work Guide

An Introduction

This guide is designed to provide material for supervisors to use at their discretion

All of the exercises provide opportunities to contribute to fulfilling the Aim that:

biology should encourage students to:

develop an understanding of scientific methods.

Candidates should be able to:

- a. devise and plan experimental and investigative activities, selecting appropriate techniques;*
- b. demonstrate safe and skillful practical techniques;*
- c. make observation and measurements with appropriate precision and record these methodically;*
- d. interpret, explain, evaluate and communicate the results of their experimental and investigative activities clearly and logically using biological knowledge and understanding and using appropriate specialist vocabulary.*

They provide the opportunity for the assessment of:

- A. Method of changing the independent variable.
- B. Method of measuring the dependent variable.
- C. Implementation of practical work.
- D. Collection and presentation of raw data.
- E. Drawing.
- F. Analysing.
- G. Drawing conclusions.
- H. Evaluating.
- I. Selecting and retrieving information.
- J. Communication.

The responsibility for safe and correct practice rests entirely with the designated supervisor, to whom the methods are offered as a source of information only.

Hazard Cards as produced by, for example CLEAPS, should be displayed wherever necessary.

CLEAPS, School Science Service, Brunel University, Uxbridge, UB8 3PH

Molecules, Cells and Systems

1.1 The cell as the basic unit of structure in prokaryotic and eukaryotic organisms

Eukaryotic cells

Practical work to include the preparation of temporary mounts and the use of simple staining techniques.

The preparation of temporary mounts, the use of simple staining techniques

Introduction

Plant and animal cells are to be studied under the light microscope. The plant cells are to be viewed by making a temporary preparation of the inner epidermis of an onion scale, stained with iodine in potassium iodide solution to make the cells more visible. A prepared slide of human epithelial cells from the lining of the cheek will also be studied.

Method

- ▼ Place 1-2 drops of iodine in KI solution in the middle of the slide.
- ▼ Take a piece of onion scale and tear it with the fingers. This should leave a very thin epidermis attached to the inside surface of the scale. Carefully remove a small piece (about 0.5 cm²) of this epidermis with the forceps, and place it in the drop of iodine solution on the slide.
- ▼ Use the mounted needle to position the piece of epidermis flat in the iodine solution, then use the needle again to lower the coverslip carefully onto the specimen, (trying not to trap too many air bubbles).
- ▼ Examine the slide under the light microscope, using the medium power (x10) objective lens, and draw 2-3 adjacent cells (at least a few cm across). Label the drawing to show the structures you can see, e.g. nucleus, cell wall, vacuole, and cytoplasm (they may not all be visible). Annotate the drawing to show the functions of the structures you have drawn.
- ▼ Calculate the magnification of the drawing:
$$\text{Microscope magnification} = \text{eyepiece lens magnification} \times \text{objective lens magnification}$$

Note this figure on your drawing.
- ▼ Now correct the magnification for the size of the drawing; that is the number of times the drawing is larger than the image seen down the microscope eg, twice the size [x2]. This gives a final magnification of:
$$\text{Eyepiece lens} \times \text{objective lens} \times 2$$

Also note this figure on your drawing.
- ▼ Use the high power (x40) objective lens to check the details you have drawn.
- ▼ Now examine the prepared slide of human cheek cells under the microscope. Locate the cells using the medium power (x10) objective lens, you will probably need a higher power to make the drawing.
- ▼ Draw 2-3 cells (not too small), label the drawing, annotate, and note the final magnification (microscope magnification x size difference).
- ▼ Make sure the drawings have suitable headings.

Teaching Notes

This practical usually works well, provided the student gets a clean piece of the onion epidermis, without any additional tissue. They also need to make sure it is flat under the coverslip, and hopefully without too many air bubbles.

The student can usually see the vacuole and cell wall clearly. They may see the nucleus, which is usually pushed over to one side of the cell by the vacuole. Occasionally it is possible to see the cell membrane in the corners, if the cells have plasmolysed slightly. Cytoplasm is usually visible as a granular material in the corners of the cells.

The cheek cells have less detail, although the nucleus can be clearly seen. Some cells may show granules in the cytoplasm.

This practical also provides an opportunity to discuss the main points of **biological drawing technique**. The important points to get across are:

Drawings should be line drawings, with no shading, or use of colour.

They should be large enough to be clear.

The lines should not be sketchy.

Pencils should be sharp, HB or H.

Label lines should be drawn with a ruler, and should not cross. The labels should be written in pencil, and should be well away from the drawing.

Labels should be annotated, either next to the label or as a suitable note, to give further information as required.

Magnification, or an indication of size/scale, should be included.

Drawings should be clearly labelled with the student's name, the name of the specimen, its classification if appropriate, type of section/squash etc, and preparation or staining details.

Sometimes, depending on the microscope and the slide, the plant cell wall cannot be clearly seen to have a thickness, but it is always drawn with a double line to distinguish it from membranes.

Requirements per student

- Microscopes
- Slide and coverslip
- Forceps
- Mounted needle
- Drawing materials
- Iodine in potassium iodide solution
- Pieces of fresh onion scale
- Prepared slide of Human cheek cells

Safety Considerations

Iodine in potassium iodide solution can be an irritant, and will stain clothes if spilled. Microscopes with adjustable mirrors must not be used in direct sunlight.

Molecules, Cells and Systems

1.1 The cell as the basic unit of structure in prokaryotic and eukaryotic organisms

Eukaryotic cells

Practical work to include the estimation of size.

The estimation of size of microscopic structures

Introduction

There are two methods you can use to measure the size of objects under the light microscope. One is a calculation based on the magnification, the other is to use an eyepiece graticule, which will need to be calibrated for the microscope using a stage micrometer. The graticule will give you a more accurate measurement, but the procedure is more complex.

Method A - Using the magnification

Make a note of the magnification of the eyepiece lens, and of the objective lens. The total magnification is found thus:

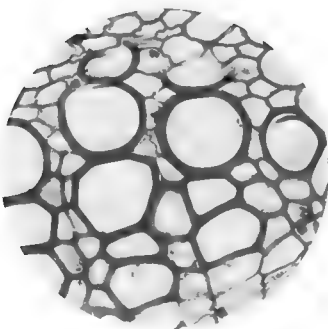
Total magnification = magnification of eyepiece lens x magnification of objective lens

The term **linear magnification** refers to the number of times an imaginary 'line' across the drawing is greater than the equivalent would be on the original specimen (as opposed, for example, to considering the magnification of the area).

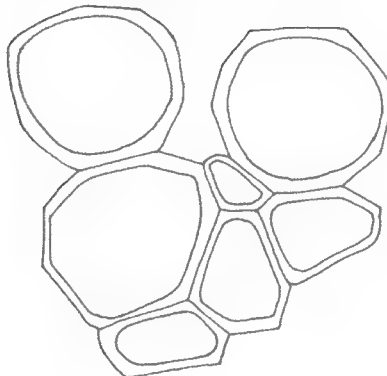
To calculate the linear magnification of the drawing, estimate how large the drawing is, compared with the view down the microscope. Look down the microscope, and then at the drawing, is the drawing x2, x3, x4 the size of the specimen?

You can then multiply the magnification by the size difference.

So if this is what you can see:
(with x100 magnification)



and this is what you have drawn:



If the eyepiece lens is x10, and the objective lens is x10, then:

Final magnification = $10 \times 10 \times 2$ (drawing is about twice the size of the specimen) = x200 magnification

To work out the size of the original object under the microscope, measure a line across the drawing in mm, and divide it by the total magnification, giving the answer in μm .

Method B - Using the Eyepiece Graticule

- ▼ To do this, you need to have a scale (graticule) in position in your eyepiece, so that it can be seen when you look down the microscope. These scales are usually on small circular pieces of glass or acetate.
- ▼ To insert the scale in the eyepiece, remove the eyepiece lens from the microscope, and carefully unscrew the top lens. If you look down into the lens body, you should see a ledge running round the sides about halfway down. Drop the scale into the lens body, so that it rests on the ledge, then replace the top lens. It does not matter if the scale is the wrong way up, but if it annoys you, unscrew the lens again and turn the scale over.
- ▼ Look through the microscope, you should see the scale overlying the specimen.
- ▼ To calibrate the scale, you need to use a stage micrometer. This can be a special slide with a scale engraved on it, or one can be made by mounting a scale on a slide under a coverslip using Canada Balsam. It usually consists of a scale 1cm long, which is divided into 100 units, each of which is 0.1 mm (100 μm), there is an extended line every 10th unit. It is worth being aware that these scales can be badly scratched.

To calibrate the eyepiece scale

- ▼ Place the stage micrometer on the microscope stage and hold it down with the clips.
- ▼ Using the eyepiece lens with the scale in, look through the microscope and focus it so you can see both scales clearly. This is usually easier if you focus the eye on the eyepiece scale and adjust the microscope so the stage scale comes into focus as well.
- ▼ Move the stage micrometer carefully so that the starting units of the two scales coincide.
- ▼ Count along the two scales until there is again a coincidence between the two scales. Note down the number of divisions along each of the two scales that this represents.
- ▼ As each division along the stage micrometer scale represents 100 μm , then:

$$1 \text{ division on the eyepiece scale (in mm)} = \frac{\text{Number of divisions on the stage micrometer scale}}{\text{Number of divisions on the eyepiece graticule scale}} \times 100$$

- ▼ At $\times 100$ and $\times 400$ magnification the lines on the scale will have a definite thickness. It is important to measure from one side of one scale mark, to the same side of the next coinciding mark.
- ▼ This procedure should be done for all the objective lenses on the microscope, so that you have a scale calibration for all magnifications. Record this information in a table with the following headings.

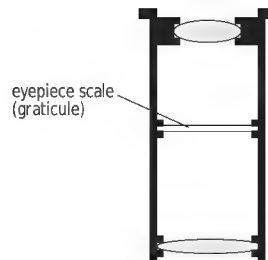
Power of lens	Magnification	Eyepiece scale 1 division (in mm)

- ▼ When you measure another specimen, you will already have the calibration figures, so all you have to do is count how many eyepiece scale divisions the specimen covers, and multiply that by the calibration factor for that objective lens.

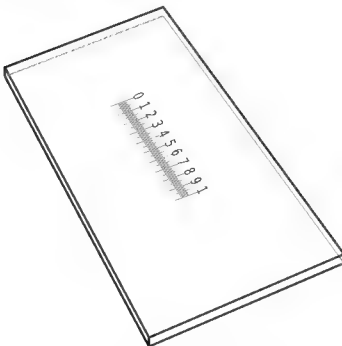
Now accurately measure as many structures as you can under the microscope using the calibrated eye piece graticule, and a range of different objective lenses.

Measurement of size under the microscope

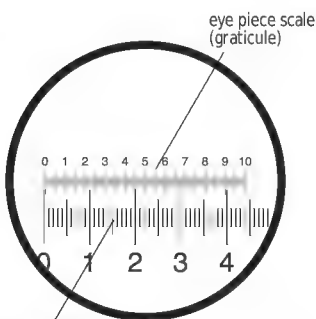
Eyepiece scale (graticule)



Eyepiece scale as seen when held up to the light away from the microscope.



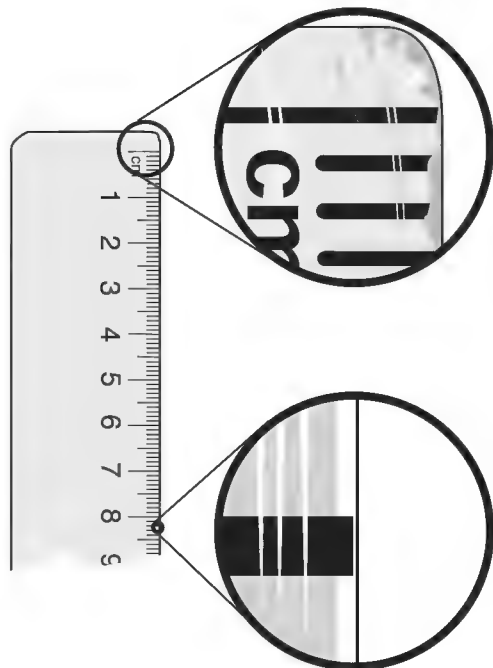
Scale in mm etched upon a microscope slide or a printed acetate sheet (slide micrometer)



slide micrometer scale

Accurate measurements can be achieved using the two scales in combination through the lenses of the microscope.

More of the object is seen under low power objective lens



Less of the object is seen under high power objective lens

Teaching Notes

Individual help is often required to ensure that students fully understand the procedure involved in calibrating the eyepiece graticule. The diameter of red blood cells at about $7\mu\text{m}$ provides a good test of their measuring technique.

Requirements per student

- Microscope
- Eyepiece graticule
- Slide micrometer
- Suitable slides for measuring

Safety Considerations

There are no special safety considerations with this practical, other than the safe use of the microscope.

Molecules, Cells and Systems

1.3 The properties of plasma membranes and the passage of substances through them

Water potential

Osmosis as the movement of water from a solution of less negative water potential to a solution of more negative water potential through a partially permeable membrane

An experiment to investigate the movement of water down water potential gradients through partially permeable membranes

Introduction

The changes in mass of tissue caused by water loss or gain, due to osmosis at the cellular level, are to be investigated. Pieces of plant food storage tissue, e.g. potato, will be placed in distilled water, and sucrose solutions of different concentrations.

Method

- ▼ Collect a set of six 50cm³ beakers, and label them 0%, 0.5%, 1%, 5%, 10%, and 15%. Put 30 cm³ of distilled water in the 0% beaker.
- ▼ Using the 15% stock solution of sucrose supplied, make up the dilutions of sucrose solution listed, and put 30cm³ of each dilution in the appropriate labelled beaker.
- ▼ Collect a potato and use the cork borer to cut out a cylinder of potato tissue. Carefully cut the cylinder into discs 1mm thick, discarding the ends with the skin on. You will need about 60 discs.
- ▼ Using a balance, weigh 10 of the potato discs, note the mass and record in a suitable table. Repeat 5 times, so you have 10 discs of known combined mass for each solution.
- ▼ Add 10 discs of known combined mass to each of the solutions. Note the time.
- ▼ After 45-60 minutes, remove each set of discs from the beakers, very carefully blot them dry on a paper towel or tissue and reweigh them. Make a note of the new masses in the table.
- ▼ Calculate the % change in mass of the potato tissue in each liquid by dividing the gain or loss in mass by the original mass and multiplying by 100. Plot a graph of % change in mass against concentration of sucrose solution, include the distilled water result.

Discuss what the results indicate about osmosis and the effect that it has on living tissue.

Use the graph to estimate which concentration of sucrose solution possibly matches the internal concentration of the potato cells.

Teaching Notes

This practical works well, and is better than the standard potato chip exercise, as the surface area / volume ratio is higher so the uptake / loss of water gives clearer results. The discs are fairly easy to cut, but to save time, the discs can be cut beforehand and kept in 5% sucrose solution.

Some students may need help calculating the volumes of sucrose solution and distilled water needed to obtain the correct dilutions.

If possible you need a balance which will show 2 decimal places to get an accurate result.

Students must be very gentle when blotting discs before reweighing

(Changes in onion ring diameter in different concentrations of solution/changes in mass of slices of banana of different ripeness/comparing change in the mass of slices of different plant materials e.g. carrot, parsnip, swede etc. are all worth a try).

Requirements per student

Cutting tile
1.5 – 2 cm diameter cork borer
Sharp knife
6 x 50 cm³ beakers
50 cm³ measuring cylinder
Syringes/pipettes
Balance
Paper towels/tissues
100cm³ 15% sucrose solution
Distilled water
Potato

Safety Considerations

The students need to be careful with the cork borer and sharp knife while preparing the potato discs.

Molecules, Cells and Systems

1.4 Large molecules in the structure and functioning of cells

Biological molecules

Carbohydrates

Proteins

Lipids

Biochemical tests using Benedict's reagent for reducing sugars and for non-reducing sugars after acid hydrolysis. Iodine/potassium iodide solution for starch. The biuret test for proteins. The emulsion test for lipids.

Biochemical tests for substances of biological importance

Introduction

A standard series of procedures are used to test for a variety of biological molecules. These tests are traditionally known as 'food tests', but here the basic test methods are performed on a standard solution of each type of substance.

Method - Benedict's test for reducing sugars

- ▼ Put on a pair of safety glasses. Collect a 500cm³ beaker and fill it about half full with tap water. Place it on a tripod and gauze over a Bunsen burner and bring the water to the boil.
- ▼ Place a small quantity of reducing sugar solution (about 1-2 cm³) in a boiling tube. Add 2 cm³ of Benedict's reagent.
- ▼ Place the boiling tube in the waterbath and boil the tube and its contents for 3-4 minutes. Remove the tube from the waterbath using test-tube tongs and place it in a test-tube rack.

If the sample contains reducing sugars, a precipitate will be produced, and the colour will change from blue through green and yellow to brick-red, depending on the amount of sugar present. This test can thus be used to give an estimation of the amount of sugar present, provided the quantities of sugar solution and Benedict's reagent used are the same each time. A small amount of sugar will turn the solution green, larger amounts yellow and considerable quantities will turn it brick-red.

Method - Benedict's test for non-reducing sugars

This test should be done if the previous test for reducing sugars gives a negative result.

- ▼ Place a fresh sample of sugar solution ($1\text{-}2\text{cm}^3$) in a boiling tube and add 1cm^3 dilute hydrochloric acid. Place the boiling tube in the waterbath and boil it for 1-2 minutes.
- ▼ Remove the test-tube from the waterbath using tongs and place it in a test-tube rack. Allow to cool for a couple of minutes. Using a spatula, add small quantities of sodium hydrogen carbonate powder, until the mixture stops fizzing. (This will probably take about 3 small spatulas full: the sodium hydrogen carbonate neutralises the acid)
- ▼ Add 2cm^3 Benedict's reagent to the boiling tube and put it back in the waterbath, using the tongs. Boil it again for 3-4 minutes.

If non-reducing sugars (e.g. sucrose) were present in the original sample, the acid will hydrolyse them to monosaccharides, which are all reducing sugars: they will then give a green/yellow/brick-red precipitate with Benedict's reagent.

Method - Samples containing both reducing and non-reducing sugars

If you suspect that a sample contains both reducing and non-reducing sugars:

- ▼ Test with Benedict's reagent and obtain the precipitate as described above (using double quantities of sample and reagent)
- ▼ Filter the tested sample through filter paper, and collect the filtrate.
- ▼ Test the filtrate with Benedict's reagent.
- ▼ Continue this process, using the filtrate each time, until you no longer get a positive result with the Benedict's test.
- ▼ Add 1cm^3 dilute hydrochloric acid to the remaining filtrate and boil it to hydrolyse any non-reducing sugars it contains. Neutralise the mixture and re-test with Benedict's reagent.
- ▼ If a non-reducing sugar was present in the original sample there will be a positive result once again.

Method - Iodine in potassium iodide solution test for starch

- ▼ Place a small sample of starch solution on a spotting tile or in a test-tube.
- ▼ Add a few drops of iodine in potassium iodide solution.

The starch will combine with the iodine to give a deep blue-black colour. This test is very sensitive, so only tiny quantities are needed. The colour will fade after a while.

Method - Emulsion test for lipids

This test uses the fact that alcohols will dissolve lipids, but water will not.

- ▼ Place a small sample of lipid in a test-tube. Add 1-2cm³ of alcohol and shake the test-tube thoroughly for a few minutes. Place it in a test-tube rack and allow it to settle.
- ▼ Collect a clean test-tube and half fill it with tap water. Carefully pour the alcohol layer from the sample into the water.

The lipids will dissolve in the alcohol as the sample is shaken. When the solution is poured into the water, the alcohol will dissolve in the water, but the lipids will come out of solution and form fine droplets. Thus if there are lipids in the sample, they will form a cloudy white emulsion in the water.

Method - Biuret test for proteins

- ▼ Place 2cm³ of protein solution in a test-tube. Add 2 cm³ of dilute potassium hydroxide solution, then add 0.5cm³ of dilute copper sulphate solution, a drop at a time. Shake the test-tube gently, then leave it in the test-tube rack for a few minutes.

If protein is present, a purple colour will develop after a few seconds (s). If there is no protein, the solution will remain very pale blue from the copper sulphate solution.

These methods may be used for testing materials in a laboratory, or for testing foodstuffs to analyse their contents.

Teaching Notes

For the emulsion test, either ethanol or iso-propanol will work, provided it is a strong solution (at least 70%).

The only problem with the standard tests is doing the emulsion test on milk, in which the emulsion effect is masked. The Sudan III test for lipids may be more appropriate for this situation, as it gives a clearer result. If milk is shaken up with Sudan III and methylene blue and allowed to settle, the red Sudan III will separate out in the fatty layer at the top, and the methylene blue in the aqueous layer at the bottom.

The test for reducing sugars can be made quantitative by carrying out the Benedict's test on a series of known concentrations of glucose solution, to produce a colour series, then testing some unknowns to identify where they fit in the series. This could be introduced as a problem for the students, to provide experience in planning experiments.

Clinistix or equivalent can be used to test for different glucose concentrations.

Once the tests are understood, the techniques could be used to analyse mixtures. This could be made more interesting by suggesting that they are testing foodstuffs for a supermarket firm who have been told that some of their foods have been spiked, e.g. try adding starch to the milk, or injecting some oil into oranges, for example.

Alternatively, there is a good variation published in *Journal of Biological Education*, (Roger Law, 1995, "An A-level food test investigation", *JBE*, Volume 29, p251-252), which uses other tests as well, to analyse mock "stomach contents" from two animals. The idea is to decide whether they are carnivorous or herbivorous. This test uses some solutions which are a bit more risky than the usual ones (Schultz' reagent for cellulose, for example.) However, it can make an enjoyable change.

Requirements per student

Boiling tubes
Test tubes
500cm³ beaker
Bunsen burner, tripod and gauze
Test-tube rack
Test-tube tongs
Spotting tile
Small spatula
Filter funnel and filter paper
Safety glasses
Small measuring cylinders or dropping pipettes

Benedict's reagent
Dilute hydrochloric acid
Sodium hydrogen carbonate powder
Iodine in potassium iodide solution
70-80% alcohol solution
Biuret 1% copper sulphate solution
Biuret 15% potassium hydroxide solution

Standard solutions of sucrose (5%), glucose (5%), Starch (1%), albumin (5%) and a small sample of cooking oil.

continued . . .

Safety Considerations

The following chemicals need to be handled carefully:

Dilute hydrochloric acid – corrosive

Iodine in potassium iodide solution – irritant

80% alcohol – highly flammable

Biuret potassium hydroxide – corrosive

Safety glasses should be worn for the Benedict's test at least: you may prefer students to wear them all the time.

The Benedict's reaction should be boiled in a waterbath – never allow students to heat the tube directly in the Bunsen flame.

Normal precautions for handling hot apparatus should be observed.

Molecules, Cells and Systems

1.4 Large molecules in the structure and functioning of cells

Chromatography

The technique of chromatography to illustrate how molecules may be separated and identified. The calculation and use of R_f value.

Two way chromatography.

The technique of chromatography

Introduction

Chromatography is a technique used to separate and analyse which molecules are present in a mixed extract from living material. It depends on the use of organic solvents, which dissolve the molecules concerned. These solutions are allowed to move up a thin layer of inert material, such as paper or a thin layer of silica gel, carrying the molecules with them. The distance the molecules move is related to the size and shape of the molecule, as well as their relative solubility in the solvent and the water associated with the layer of inert material.

For each combination of molecule and solvent, the distance moved relative to the solvent alone is characteristic, and can be used to identify the molecule. The value is known as R_f (Relative Front) and is calculated thus:

$$R_f = \frac{\text{Distance moved by molecule}}{\text{Distance moved by solvent}}$$

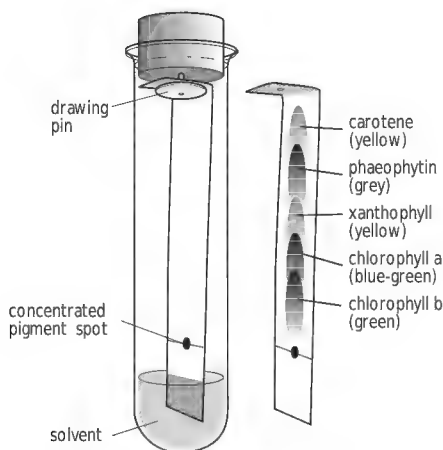
Method

- ▼ Cut a piece of chromatography paper (or filter paper) about 1.5cm wide, and long enough to reach the bottom of the boiling tube. Fold the top 1cm over, and make sure the paper will fit into the boiling tube, with the folded edge at the top, the end nearly reaching the bottom, and the sides NOT touching the sides of the tube. If necessary, trim it to fit.
- ▼ Rule a pencil line (not pen!) across the paper about 2.5 cm from the bottom. Mark the centre of the line with a cross. Write your name at the top of the strip in pencil.
- ▼ Collect a handful of plant material, and cut it up quite finely with a sharp knife. Place 5 cm³ of propanone in the mortar, and grind up the plant material in the mortar, using the pestle and a small amount of sand, (or use a liquidiser). The green pigments should dissolve into the propanone. As you grind up the plant material, remove any tissue that is well ground, and add more, but do not add any more propanone: you need to get as concentrated a solution as possible.
- ▼ Once you have a concentrated solution, filter the contents of the mortar through a piece of gauze in a filter funnel. Discard the solid materials.
- ▼ Put a small amount of solvent (to a depth of not more than 1.5 cm) in the bottom of the boiling tube. Put the bung in the tube and leave it on one side so that the air in the tube can saturate with the solvent vapour.

continued

- ▼ Using a piece of very fine capillary tubing or the head end of a pin, place a drop of the extract on the cross in the middle of the pencil line of the paper strip. Use a hair dryer to dry the drop, then put another drop in the same place. Repeat this process until you have a small area of concentrated pigment. Be patient with this: you will get a much better result if you let each drop dry properly before you add the next. You should add at least 5-6 drops.
- ▼ Use a drawing pin stuck into the base of the bung, or a split bung, to suspend the paper in the boiling tube, so that the end of the paper reaches into the solvent, but the spot of pigments is not in the solvent. Adjust the amount of solvent if necessary.
- ▼ Leave the boiling tube in a rack to allow the solvent to move up the filter paper. When the solvent has nearly reached the top of the strip of paper, remove the bung and detach the paper. Draw a pencil line on the paper to mark the level reached by the solvent, then leave it on the bench (or in a fume cupboard) to dry.
- ▼ Measure the solvent distance, and the distances moved by the spots of pigment. Identify the pigments by their colour and R_f values, using the information given in the table below. If you have produced a good concentration of pigments in the extract and spot, you should be able to identify all five pigments.

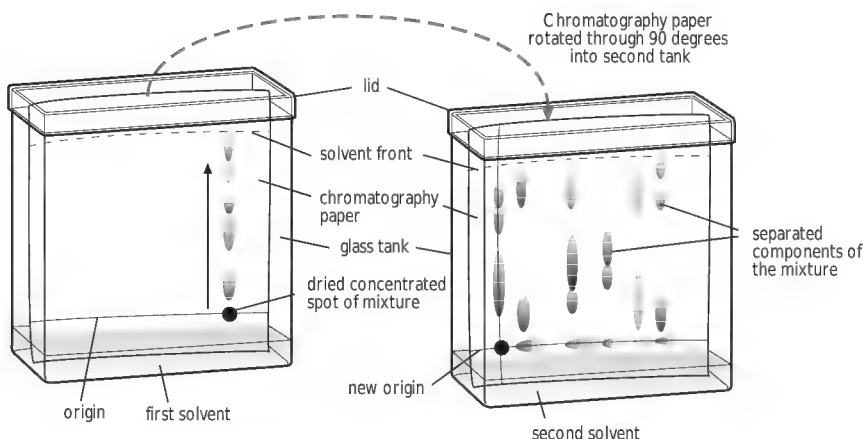
Pigment Name	R_f value	Colour
Carotene	0.95	Yellow
Phaeophytin	0.83	Yellow-Grey
Xanthophyll	0.71	Yellow-Brown
Chlorophyll a	0.65	Blue-Green
Chlorophyll b	0.45	Green



Method - Two way chromatography

Two way chromatography is used to make further separations of substances that may not have been separated sufficiently on the first run of a chromatography investigation.

The chromatography paper from the first run is turned by 90° and re-run as shown in the diagram. Details are provided with Chromatography kits, and are not repeated here as no new principles are involved.



Teaching Notes

The solvent is made from 1 part 90% Propanone (Acetone) to 9 parts Petroleum ether (alternatively 12 parts: 100 parts)

The plant material needs to be as green as possible! Nettle leaves work well, and can be handled easily with gloves. Alternatively, spinach leaves also give a good result. As a possible extension, or for a student project, it might be interesting to try a different coloured leaf, such as red cabbage, although the R_f values for any extra pigments may be difficult to find.

One important factor in getting the practical to work well is to get the chlorophyll extract as concentrated as possible: the students need to spend time over this, and grind up quite a lot of plant material in as little propanone as they can. The other stage at which patience is needed is the preparation of the pigment spot. They must let each drop dry thoroughly, before adding the next, and they need to add at least 5-6 drops. Using a hair dryer speeds up the process. To keep the evidence of the experiment; after allowing the filter paper to dry, it should be sealed under Sellotape so the pigments do not fade.

If you have access to thin layer plates, they tend to work better than paper sheets, but are more complex to set up. If you do use thin layer plates, the R_f values will be different from those given here.

Requirements per student

Filter or chromatography paper
Scissors
Boiling tube with a rubber bung
Drawing pin
Ordinary pin or piece of fine capillary tubing (especially fine chromatography droppers are available)
Pestle and mortar or liquidiser
Sand for grinding
Pencil and ruler
Sharp knife
Cutting tile
Filter funnel
Piece of gauze, about 12 cm square
50cm³ beaker
Test-tube rack
5cm³ propanone
10cm³ solvent
Plant material, e.g. nettles, spinach etc.

Safety Considerations

Both the propanone and the solvent are highly flammable and need to be handled with care. The lesson should be run in a well-ventilated room, and all naked flames should be extinguished. Ideally the strips should be dried in a fume cupboard.

If you use nettle leaves, they should be handled wearing disposable rubber gloves of the surgical type or ordinary rubber gloves, these will be better than the very thin film gloves. Once crushed or cut up, the nettles can be handled with bare hands.

Molecules, Cells and Systems

1.5 Enzymes are proteins which control biochemical reactions in cells

Factors which affect enzyme activity

Description and explanation of the effects of temperature, pH, substrate and enzyme concentration on enzyme activity.

An experiment to investigate the effect of temperature on the activity of catalase

Introduction

Catalase catalyses the breakdown of hydrogen peroxide to oxygen and water.



The rate of production of oxygen can be measured, and gives a measure of the rate of the reaction.

Catalase is produced in cells to prevent the accumulation of hydrogen peroxide, which is toxic.

In this experiment potato tissue is used as a source of catalase.

The effect of temperature on the rate of this reaction will also be investigated.

TAKE CARE with the HYDROGEN PEROXIDE - wear safety glasses and try not to get any on the skin, wash it off immediately if you do.

Read the method sheet through carefully before you start.

Method

▼ There are various pieces of apparatus that you can use to collect and then measure the volume of oxygen gas that will be evolved when the catalase in the potato tissue reacts with the hydrogen peroxide.

a The oxygen gas can be collected over water in an inverted measuring cylinder.

b The oxygen can be collected over water in the inverted barrel of a 20 cm³ syringe.

c The oxygen can be collected and measured in a manometer tube filled with coloured fluid, linked to the reaction vessel. You will be advised which method(s) to use.

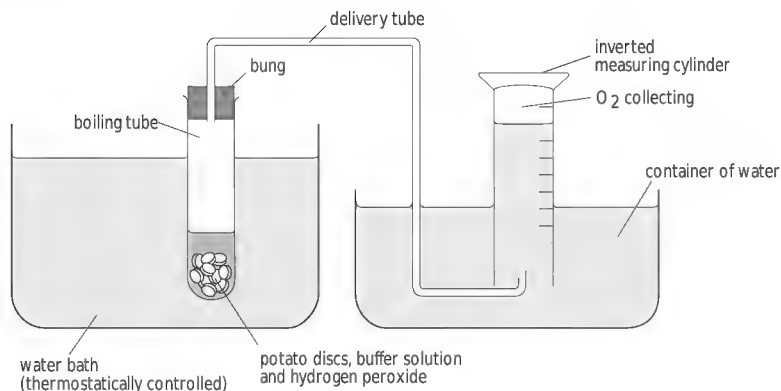
The details below relate mainly to the 'inverted measuring cylinder' method.

▼ Using a cork borer a little smaller in diameter than the boiling tubes, cut some long potato cylinders. Then using a knife (scalpel) carefully cut the potato cylinder into discs about 1 mm thick.

▼ Place the discs into a few cm³ of pH7 buffer in a watch glass to keep them moist. You will need 50-60 discs.

▼ Using a boiling tube as the reaction vessel, place about 10 potato discs into the tube and just cover them with a measured volume of pH7 buffer.

continued



- ▼ Place the tube in a water bath, at the first temperature you intend to use, e.g. 20°C and allow the discs time to reach the correct temperature. Place a boiling tube of hydrogen peroxide into the water bath at the same time, so it also reaches the same temperature.
- ▼ Assemble the apparatus; using a rubber bung with a delivery tube inserted, and check that this will fit under the inverted 25 cm³ measuring cylinder full of water in its container of water. You may have to use a clamp stand to support the cylinder.
- ▼ When you are ready, measure 5cm³ of hydrogen peroxide (H₂O₂) in a syringe. Carefully loosen the bung of the boiling tube, and very quickly and safely add the peroxide. Replace the bung and start the stopclock, tap or shake the tube if necessary to mix the contents.
- ▼ Record the volume of oxygen produced every 30 s for 3 minutes.
- ▼ Remove the boiling tube, wash it out and add 10 new discs and the same volume of pH7 buffer as used before. Put the tube back in the water bath and carefully raise the temperature by 10°C.
- ▼ If necessary refill the measuring cylinder with water.
- ▼ Add 5cm³ hydrogen peroxide to the boiling tube, quickly replace the bung, start the stopclock and take the readings as before.
- ▼ Repeat the experiment at 30, 40, 50 and 60°C using a new set of potato discs each time.

Look at the rate at which the oxygen has been produced during each 30 second period. Can you explain the results in terms of an enzyme reacting with its substrate?

Calculate the mean amount of O₂ produced during the 3 minute period, at each temperature. Plot a graph of these figures against temperature.

How could you improve the method to obtain more reliable results.

Plan how you could adapt this experiment to investigate the effect of pH, substrate concentration, or enzyme concentration, on the action of Catalase. Check the new plan with the supervisor, then carry out the investigation and write a full report, including analysis of results, conclusion, evaluation etc.

Teaching Notes

This practical usually works quite well and it is a good exercise for students to demonstrate their manipulative skills. A number of methods can be used in this experiment to measure the volume of oxygen produced, as mentioned on the student sheet: e.g. over water in an inverted measuring cylinder, over water in the barrel of a syringe (a small piece of rubber tubing is attached to the narrow end of the syringe which has a screw clip so the barrel can be filled with water) or in any simple manometer.

A bit of juggling with the number of potato discs and the volume of peroxide (or its concentration) may be necessary to achieve a good result. But students using the inverted measuring cylinder method at 40°C recently obtained results of:

O₂ produced: 30s/5 cm³, 60s/2cm³, 90s/1.5cm³, 120s/1.0 cm³, 150s/0.5 cm³
180s/0.5 cm³.

Hydrogen peroxide can be added from a syringe, the needle of which is inserted through the cork, to improve accuracy.

If the students are going on to measure the effects of pH, they will need to be supplied with quantities of buffers: pHs suggested are 4, 5, 6, 7, 8, 9. They will need only a few cm³ of each buffer per student.

Substrate concentration can be varied using different concentrations of hydrogen peroxide, such as 5 vol., 10 vol., 15 vol., and 20 vol.

Effect of enzyme concentration can be measured by varying the number of potato discs used. Alternatively you could vary the size of the discs (either diameter or thickness). It is quite important that the discs are cut to the same sizes for this version of the experiment. This would also allow them to consider the effect of surface area: volume ratio.

Requirements per student (measuring cylinder method)

Boiling tube
Large beaker (500 cm³)
Bunsen burner
2 tripods and gauzes
or thermostatically controlled water bath and 1 tripod and gauze
Bung with delivery tube
Measuring cylinders 25 cm³ and 10 cm³
Trough or equivalent
Cork borer 10mm diameter (No6)
Watch glass
2 100cm³ beakers
5 cm³ syringe or dropping pipette
1 clamp stand
Stopclock
Thermometer
Knife/scalpel
50cm³ pH 7 buffer
50cm³ 20 volume hydrogen peroxide
Potato

continued

Safety Considerations

Use of the sharp knife and cork borer need care.

Hydrogen peroxide is an oxidant, and needs to be kept away from heat and stored in a fireproof cabinet.

Hydrogen peroxide can also be an irritant or corrosive at strong concentrations, so care is needed especially with the 15/20 volume.

Safety glasses, overalls and disposable gloves should be worn and contact with the skin avoided.

Molecules, Cells and Systems

1.6 Tissues and organs

Epithelial tissue

The essential features of the alveolar epithelium as a surface over which gas exchange takes place

Blood

Recognition of red blood cells, lymphocytes, monocytes and granulocytes

Blood vessels

The structure of arteries, arterioles and veins in relation to their function

Microscopic examination of tissues and organs

Introduction

The observation of lung tissue can be problematic with much loss of structure occurring in the sectioning process; many of the air tubes and blood vessels are cut obliquely in section, further obscuring the 'text book' structure. Blood smears similarly pose problems, mainly because of the small size of the red blood cells and the infrequency of some types of white blood cells. The structure of arteries and veins is usually clear, although the thin walled veins can be mistaken for large spaces in the tissue. It is not possible at this level to identify arterioles in cross section with any certainty.

Method (i)

- ▼ Examine the prepared slide of a cross section (TS) of lung tissue showing alveoli under the microscope using the medium power (x 10) objective lens and then the high power (x40) objective lens.
- ▼ Both the alveoli and the blood capillaries are lined with very thin squamous epithelial cells, which lie so close to each other that it may be very difficult to distinguish between them. Even with a very good light microscope the stained nuclei of the squamous cells may be the only cell structure you can identify.
- ▼ The capillaries (in which the squamous lining is called the endothelium) contain red blood cells, also cut in section, and these should be visible.
- ▼ Identify alveolar epithelial cells, and pulmonary capillaries containing red blood cells, draw a small portion of what you can see, enlarging it as much as you can.
- ▼ Label the diagram, annotate it in relation to gas exchange, and give it an appropriate heading.
- ▼ Record the final magnification (eyepiece x objective x size difference).

continued

Method (ii)

- ▼ Examine the prepared slide of a human blood smear under the microscope using medium power ($\times 10$) objective lens, and then high power ($\times 40$) objective lens.
- ▼ Identify the various types of blood cell: red cells, lymphocytes, monocytes and granulocytes. (You may need to look at a number of fields of view.) The nuclei of the white cells stain purple and therefore show up quite clearly.

Granulocytes 72% (Lobed nucleus and granular cytoplasm)
Lymphocytes 24% (Large circular nucleus fills most of the cell).
Monocytes 4% (Larger cell with large kidney shaped nucleus).
- ▼ Draw examples of each type of cell and then label and annotate the drawings with details of their functions. Give your drawings a heading.
- ▼ Record the final magnification of the drawing (eyepiece $\times$ objective $\times$ size difference).

Method (iii)

- ▼ Examine the prepared slide showing a section through an artery and vein under the microscope, using the magnification you find most suitable.
- ▼ Draw a plan of each vessel, showing only layers of tissues (no cell detail), label the drawing, and give it an appropriate heading.
- ▼ Record the final magnification of the drawing (eyepiece $\times$ objective $\times$ size difference).
- ▼ Draw a suitable table and complete it to compare the structural similarities and differences between arteries, arterioles, and veins.

Teaching Notes

Details of alveoli and capillaries may be difficult to identify even with help. You may decide that a drawing is not necessary (or possible).

The students sometimes have difficulty in focussing on the red blood cells under high power.

The nuclei of the leucocytes are stained purple; the cytoplasm is usually pale, with purple, blue or pink stained granules. They are usually easier to spot under the $\times 10$ objective lens. The student can then move to a higher power to draw the cells.

The different types of white cell: lymphocytes, monocytes and granulocytes are found in the following proportions (as % of total white cell count)

Granulocytes	72% (Neutrophils 70%, Eosinophils 1.5%, Basophils 0.5%)
Lymphocytes	24%
Monocytes	4%

The blood vessels are usually straightforward to identify and label, although they may not be able to differentiate the various layers of the wall.

Requirements per student:

- Microscope
- Prepared slide of T.S. alveoli
- Prepared slide of Human blood smear
- Prepared slide of T.S artery and vein
- Drawing materials
- Reference books/photos etc.

Safety Considerations

There are no safety considerations for this practical, other than the safe use of the microscope.

Molecules, Cells and Systems

1.7 The blood system as a mass flow system linked with exchange surfaces

Ventilation

The composition of inhaled and exhaled air

The composition of inhaled and exhaled air

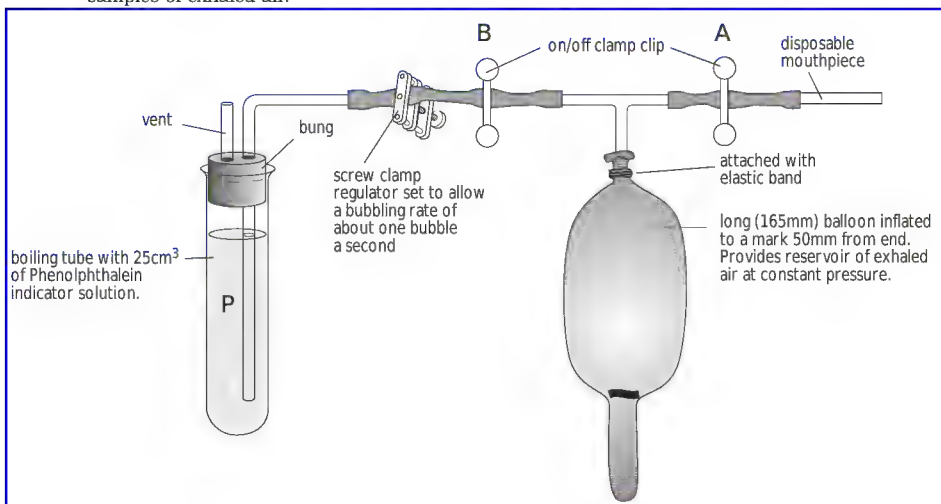
Introduction

The consumption of oxygen and the production of carbon dioxide results from aerobic respiration in body cells.

Both can be measured, but the measurement of oxygen consumption is quite difficult, whereas the measurement of the increase in carbon dioxide in exhaled air is much simpler.

Method

This investigation can be used to make simple quantitative measures of CO₂ in samples of exhaled air.



- ▼ Close tap B and open tap A.
- ▼ Inflate the balloon in one breath up to the mark and close tap A.
- ▼ Open tap B and allow air to bubble through the alkaline indicator solution (P). CO₂ being an 'acid' gas will neutralise the solution P. Count the bubbles and record the time for the pink colour to disappear.
- ▼ The ratio:

$$\frac{\text{Time to decolour P at rest}}{\text{Time to decolour P after a period of exercise}} = \text{CO}_2 \text{ Index}$$
- ▼ CO₂ Index and pulse rate can be plotted against time on the same graph.

Teaching Notes

Time needs to be taken to establish a constant bubbling rate.

This involves inflating the balloon to the same extent each time, and having the screw clip adjusted correctly.

Requirements

Boiling tube with 25cm³ of phenolphthalein indicator solution.

500 cm³ of phenolphthalein indicator solution prepared by dissolving 1g of solid phenolphthalein in 500 cm³ of distilled water and adding sodium hydroxide drop by drop until a pink colour is obtained.

Long balloon with a mark to establish a standard volume and pressure of inflation.

The apparatus as illustrated

Disposable mouth pieces

Safety Considerations

The subject should be in good health and no pressure must be exerted on the subject to take part in any test activity, neither should any element of competition be allowed to enter the proceedings.

It is advisable to ask the parents/guardians of the students to complete a prior consent form (or the students themselves if they are over 18) before asking them to be subjects for experiments: a sample copy is provided.

Supplementary Practical Work Sheet

Molecules, Cells and Systems

1.8 The functioning of the heart in relation to the level of activity of an individual

Effects of exercise

Changes in cardiac output and pulmonary ventilation with exercise.

The effects of exercise on the pulse rate and breathing rate

Introduction

The heart and breathing rate both increase during exercise to supply the muscles with extra oxygen and glucose, and remove carbon dioxide.

It is not easy to measure the changes in cardiac output and pulmonary ventilation with exercise without certain equipment. Cardiac output is the product of the heart rate and the stroke volume, and pulmonary ventilation is the product of the rate and depth of breathing. In this simple investigation only heart rate and breathing rate will be measured.

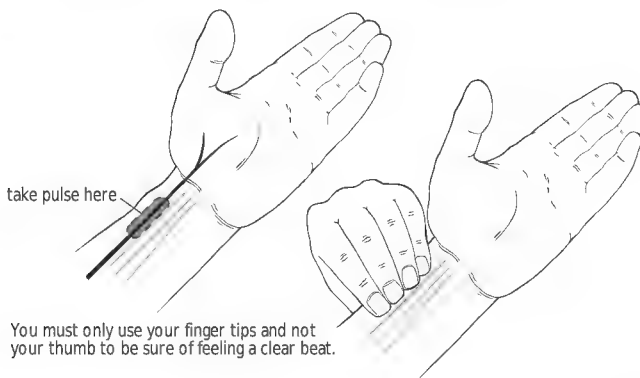
Groups of four allow: subject A to exercise, B to check pulse, C to check breathing, and D to time and record.

The pulse rate is the product of the heart rate, and can be taken to be the same thing. As the ventricles contract blood is forced out into the arteries, the walls of which expand to accommodate the surge of blood from the heart. When the ventricles relax the elastic walls of the arteries recoil until the ventricles contract again in the next cardiac cycle.

Method - Measuring the Resting Pulse Rate and Resting Breathing Rate

The pulse can be felt in the radial artery at the wrist as shown.

Breathing can be counted by placing a hand on the subject's back just below the shoulder. One inhalation and one exhalation counting as one complete breath.



continued

- ▼ Allow the subject to settle for two minutes (whilst standing).
- ▼ During this time locate the pulse in the left wrist as illustrated (do not use your thumb).
- ▼ After 2 minutes count the pulse beats and breaths for 30 s, and record the results in a suitable table.
- ▼ It is possible to count for a shorter period e.g. 6 s, but when measuring the resting pulse rate the longer the counting time, the more accurate is the conversion to beats per minute (*bpm*)
- ▼ At the end of counting, leave the subject at rest for a further 2 minutes (whilst standing).
- ▼ Repeat the 30 second pulse count/breathing procedure, and record the results.
- ▼ Allow to rest for 2 minutes (whilst standing).
- ▼ Repeat the 30 second pulse count/breathing procedure, and record the results.
- ▼ Convert all results to pulse beats per minute or breaths per minute and work out the averages.

Sample Method -

Determining the effect of exercise on the resting pulse rate and resting breathing rate

- ▼ Subject A exercises at a steady rate for 3 minutes. This exercise can be using an exercise bike, or jogging on the spot, or stepping up and down using a box or the stairs. It is important that the exercise should be at a steady rate, which is one that the entire group can keep up with.
- ▼ Immediately after the exercise, B should count subject A's radial pulse rate. Student C should check the breathing rate at the same time, while D keeps track of the time and records the results. Count both pulse rate and breathing rate for 30 s, then multiply the results by 2. Subject A should not try to count either pulse or breathing themselves, as this can influence the rates.
- ▼ Continue to count pulse rate and breathing rate for 30 sec. of each minute for four minutes, while subject A rests (standing). Do not stop the clock between measurements, by counting for only 30s you should have time to record the results for each minute before you start the next.
- ▼ Change roles within the group, and repeat the process (steps 2-5) for each student in the group, so you have all exercised, and recorded results etc.
- ▼ If you have time, repeat the whole process again for all members of the group, to check the reliability of the results.
- ▼ It is also relatively easy to sample exhaled air at various intervals throughout the procedure and to measure the increase in carbon dioxide production with exercise. A procedure is described previously (p27 - 10.7).

Present the results in an appropriate way, and in the report consider the different results from members of the group. See if you can relate the results to different levels of fitness.

Evaluate the evidence and the method.

Teaching Notes

Generally the resting pulse rate is taken when the subject is flat on their back. However, where the intention is to investigate the effect of exercise on the pulse rate, it may be better to measure the 'resting' pulse rate standing, otherwise there is an increase in the pulse rate associated with getting up to participate in the exercise.

The breathing rate can be obtained by resting one hand lightly on the subject's back, just below the shoulder.

In recent years, electronic heart rate monitors (HRM's) have become more readily available. Clear advantages offered by HRM's over manual pulse-taking are that heart rate can be continuously measured over extended periods of time, and also during a wide range of physical activities which do not lend themselves to periodic bouts of wrist holding. The range of the transmitter is about 1metre; this is not a great distance but it does not usually create any practical problems. The receiver, depending on the model and the cost, may give a simple digital display of current heart rate, or it may be capable of storing heart rate data in a number of files which can be retrieved either manually or down-loaded to a PC.

To investigate the effects of exercise on the body it is necessary to establish a standard rate of exercise, during and immediately after which it is possible to record various measurements of the effect of exercise.

The validity and reliability, and relative safety of sub-maximal and maximal tests should be understood.

The ability to extrapolate from sub-maximal results to maximal results, and the correlation between various measures of body response to exercise, will all ensure a scientific approach to the investigation.

A cycle ergometer, heart rate monitor, and stethograph allow accurate and reliable recording, but are beyond the reach of many. For those without access to these, the Step Test, along with simple measuring of e.g. heart rates (pulse rates), breathing rates, temperature, and even composition of expired air (procedure described earlier) can still produce reasonably valid and reliable results.

Sub-maximal tests like the Step Test pose less risks for the subjects and the responsible supervisor than do maximal tests like the shuttle run, or those that can be performed on the cycle ergometer.

The step test involves stepping up and down a measured height at a controlled rhythm, requires the minimum of apparatus and space, and can be administered to large numbers at the same time. The stepping height can be adjusted for individuals, for example from between 10 cm and 50 cm, depending upon the height and weight of the subject. The stepping rhythm is established with the aid of a pre-recorded signal, of so many steps per minute (*22 for females and 24 for males*). There is always the risk of stumbling, so care must be taken.

The pulse rate and breathing rate can be taken before, during and after a standard stepping period (*usually 3 or 5 minutes*). Once the heart rate has returned to its pre-exercise resting rate, the work period can be repeated if necessary with increased effort, for example by increasing the mass with added weights, and/or the rate of stepping.

continued

Step tests are theoretically as valid (*they should measure what they claim to measure*) and reliable (*they should do so consistently*) as cycle ergometer tests. However, they rely on doing work by raising the body weight against gravity at a set rate, and their validity and reliability depends on correct stepping technique which is difficult to maintain. Any change in the height due to poor leg extension, or in the rate of stepping during a test, will change the work rate, usually reducing it, and this will invalidate the results. Stepping efficiency is also affected by the length and proportions of the legs.

SYKES, K. (1987) *Fitech Step Test*. Fitech Ltd. Alan House, 17-19 Boughton Road, Chester, CH3 5AE.

Also see: HEYWARD, V. H. (1991) *Advanced Fitness Assessment and Exercise Prescription*. Human Kinetics Books, Champaign, Illinois.

This can be a straightforward practical exercise, which can give some interesting results, with a minimum of apparatus needed; especially if the age, sex or level of fitness varies within each group.

You need to make sure students are checking pulse and breathing rate correctly, and that they have made the practical as valid and reliable as possible, with equal levels of exercise, etc.

Requirements per group of 4

Step boxes, bench, or safe stairs, or exercise bike
Stopclock

Safety Considerations

The subject should be in good health and no pressure must be exerted on the subject to take part in the test activity, neither should any element of competition be allowed to enter the proceedings.

It is advisable to ask the parents/guardians of the students to complete a prior consent form (or the students themselves if they are over 18) before asking them to be subjects of investigations: a sample copy is provided.

Sample Prior Informed Consent Form

Title of Investigation/Experiment:.....

Objectives:.....

Description of Procedure:.....

Questions: All questions relating to the investigation or experiment will be answered in full.

Safety: All the procedures to be used in this investigation/experiment are widely and commonly used in Schools and Colleges, and although all activity involves some risk of an accident, the risk of harm in this investigation/experiment is no greater than in normal everyday life, or than in a supervised practical lesson.

Right of Withdrawal: You are absolutely free to withdraw from the investigation/experiment at any time. If during the investigation/experiment you feel in any way uncomfortable and wish to stop, you should do so.

Reassurance of Confidentiality: All information obtained as a result of the investigation/experiment will be treated confidentially.

Availability of Results: The data may be recorded manually, and/or stored on a computer, but not in association with your name. You are entitled to a copy of your own results on request.

Use of Results: The data may be used in discussions and/or as part of assignments or projects, but not in association with your name. They will only be used to illustrate general physiological principles, and will not be used for diagnostic or any other purposes

Statement of Consent: I fully understand the nature of the tests, and agree to participate in this investigation/experiment. To the best of my knowledge I suffer from no illness or condition that will:

i prevent me from successfully completing the tests,

ii be made worse in anyway by my taking part in the tests.

Name of Subject:.....

Signed:.....

Date:.....

Name of Parent or Legally Authorised Representative:

.....

Signed:.....**Date:**.....

Applied Biology

2.1 Enzyme isolation from microorganisms and applications in biotechnological processes

Application of enzymes in biotechnological processes

Experiment to investigate the action of Immobilised Lactase enzyme on Lactose in milk

Introduction

Immobilised enzymes and cells are used increasingly in biotechnology, as they allow easier processing and harvesting of products. The enzyme or whole cell preparation is mixed with a solution of a gel based on alginate or a similar polymer, usually extracted from seaweed. One they are mixed, the gel is treated to make it set. The beads or mesh can then be used in a column or similar flow system.

Using enzymes in this way has a number of advantages:

The beads are simple and rapid to produce; the process can be carried out under sterile conditions if necessary, so production can be kept clean

The process is gentle on the enzymes, causing little damage

It stabilises the enzymes, making them more resistant to heat, pH changes etc.

The enzymes can be used and re-used for long periods

Harvesting is simple, as the beads can be removed from the substrate and products easily, for example by sieving

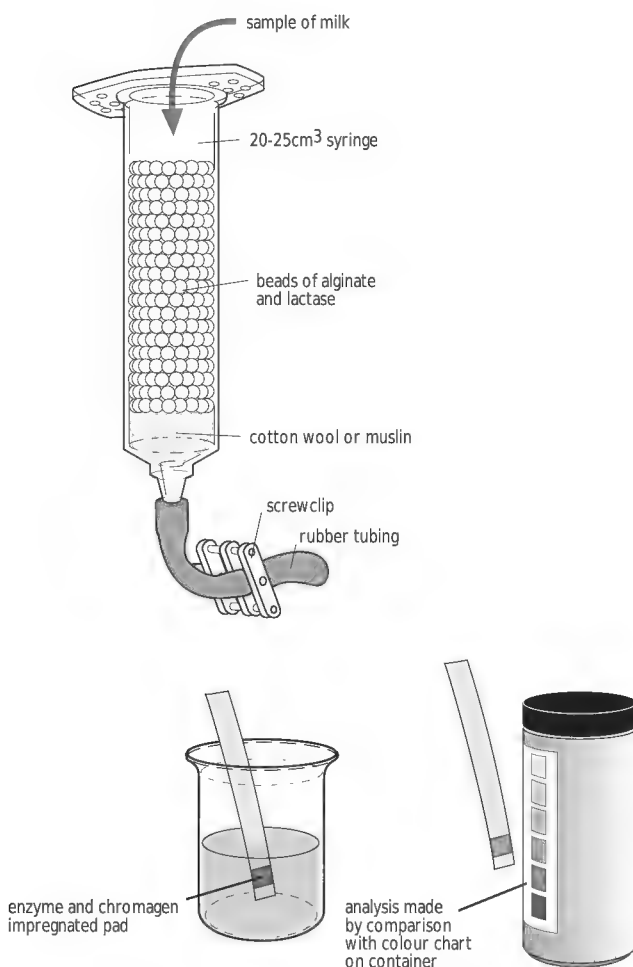
If the beads or mesh are placed in a column system, a continuous flow process can be set up, which makes production more economic.

Method

- ▼ Collect 20 cm³ of 2% sodium alginate solution.
- ▼ Stir it with a glass rod to ensure an even consistency.
- ▼ Dissolve 1.4g calcium chloride in 100 cm³ distilled water.
- ▼ Add 2 cm³ of lactase solution to the alginate gel, and stir to mix.
- ▼ Draw some of the alginate mixture into a syringe. Expel it drop by drop into the calcium chloride solution, from a height of about 10 cm: this will allow it to form beads.
- ▼ Leave the beads to stand in the calcium chloride solution for 10 minutes, to allow the beads to set completely.
- ▼ Drain the beads through a sieve or tea strainer and rinse with tap water.
- ▼ Place a small piece of cotton wool or muslin cloth in the bottom of a 20 cm³ syringe (or larger) to prevent the beads blocking the nozzle. Place a short piece of rubber tubing over the nozzle of the syringe and put an efficient screwclip over the tubing. This will allow you to control the flow of milk through the column.

continued

- ▼ Support the syringe in a clamp stand, nozzle downwards, and fill it with the alginate beads containing lactase, to within 1cm of the top. (See diagram)
- ▼ Collect a 50cm³ sample of fresh milk, and transfer 5 cm³ into a test tube. Label it 'No run' and put the test tube in a rack. Pour the rest of the sample slowly through the column, collecting the treated milk in a clean 50cm³ beaker at the bottom.
- ▼ Take a 5cm³ sample of the treated milk, put it in a test tube and label it 'Run 1'. Slowly pour the rest of the milk through the column again, and collect in a beaker.
- ▼ Take a new 5cm³ sample, label it 'Run 2', and repeat the process once more. Collect a final sample of treated milk and label it 'Run 3'.
- ▼ Test each of the four milk samples you have taken with a Clinistix strip, to measure the quantity of glucose in each of the samples.



Teaching Notes

The alginate solution and the calcium chloride solution can be prepared in advance, so the students can start making the beads immediately. Also, 3-4 sieves will usually be enough for a whole class.

The lactose solution is quite expensive, but it needs to be used neat, as supplied, or the enzyme reaction is very slow. It can be obtained from biological suppliers, or from the National Centre for Biotechnology Education, University of Reading, Whiteknights, Reading RG6 2AJ.

This practical works well, and the students usually enjoy making up the beads. A similar process can be used to prepare other immobilised enzymes e.g. sucrase (invertase), or whole cell samples e.g. yeast.

This experiment would make a good basis for a student project, for example trying different quantities of enzyme, or different sizes of beads to get the optimum efficiency in a simple column. They could also try different temperatures or using different pH-buffered solutions, to explore the improved stability of immobilised enzymes over non-immobilised ones.

Requirements per student:

Glass stirring rod
Sieve or tea strainer
5 or 10 cm³ syringe
20 or 25 cm³ syringe with rubber tubing and screw clip
Muslin
Clamp stand
4 test tubes
Test tube rack
1 x 250 cm³ beaker
2 x 100 cm³ beakers
3 x 50cm³ beakers
Labels or marking pen
20 cm³ of sodium alginate solution
1.4g calcium chloride
2cm³ Lactase solution (as supplied).
Distilled water
4 Clinistix (or similar) test sticks for glucose
50 cm³ sample of fresh milk

Safety Considerations

Unless the practical is carried out in a food preparation area, with equipment used exclusively for food preparation, it is best not to allow the students to taste the milk preparations.

(After the National Centre for Biotechnology Education)

Applied Biology

2.2 Genetic information in cell division

Mitosis

Use of appropriate staining techniques in the study of mitosis in suitable plant material

Preparation of an onion root tip squash to show stages in mitosis

Introduction

An onion root tip is to be used to show the stages of mitosis in the dividing cells. The tip will need to be softened so the cells can be squashed to spread the chromosomes in one plane, and the chromosomes stained to see the stages of mitosis.

Method

- ▼ Remove a root from a growing onion bulb. Carefully cut about 0.5cm from the tip of the root, making sure it is the tip end, not from the end near the onion. (It should be slightly pointed).
- ▼ Place the root tip in a watch glass. Add 9 drops of acetic orcein stain, and 1 drop of 1 molar hydrochloric acid. Heat the watch glass gently, using a hotplate or spirit burner, or by passing it carefully but quickly through the flame of a bunsen burner: DO NOT LET IT BOIL! Keep the watch glass hot but not boiling for 5 minutes: if you have to hold it, use forceps.
- ▼ Place the root tip on a clean slide, and add a drop of 45% ethanoic acid. Cut away all but the terminal 1mm. Cover with a clean coverslip.
- ▼ Squash the root tip gently using a pencil: hold the pencil vertically just above the coverslip, then let it fall through the fingers onto the coverslip. Repeat this 2-3 times. This should spread the cells out under the coverslip so they can be examined under the microscope.
- ▼ Examine the slide under the microscope, using the medium power (x 10) objective lens, then high power (x40) objective lens. Identify the various stages of mitosis, and make annotated drawings of each stage.
- ▼ Record the final magnification of the drawing (eyepiece x objective x size difference)

Teaching Notes

Growing the onion roots needs to be started 6-7 days before the practical. It is best to use onion sets from a garden centre, or locally grown onions, as some onions bought from a supermarket have been treated to prevent sprouting.

Push two cocktail sticks through the onion at right angles: these will support the onion in the beaker, as shown in the diagram. Fill the beaker with water until it just covers the root area of the onion, and leave it in a cool place until the roots have sprouted. They do not have to be kept in the dark. Ideally, the roots need to be about 2 - 5 cm long.

If the onions are ready before the lesson is due, or if you wish to harvest onion roots and store them until needed, they can be treated with Carnoy's solution (6 parts absolute ethanol: 3 parts chloroform: 1 part glacial acetic acid) for 24 hours, then stored in 70% ethanol until needed.

The watch glasses need to be heated gently for five minutes, but they should not be allowed to boil or dry out. If they begin to dry out, an extra drop of stain can be added.

If the students have trouble finding mitotic cells in their specimen, check that they have actually used the root tip: if you can see developing xylem or phloem, they have retained the wrong portion.

It can be useful to have a few prepared slides of root tip squashes or sections, in case the students have difficulty with the slide preparation. Appropriate photographs and / or diagrams will also be useful. In addition, it will be useful if the students can see video footage of mitosis. It is important to make it clear that mitosis is a continuous process, which is subdivided for convenience.

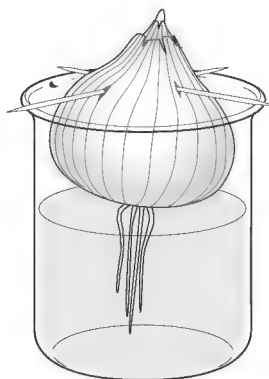
It might be possible for the students to estimate the relative length of each stage of mitosis, by counting how many cells they can see which are in each stage.

Requirements per student

Watch glass, forceps, scalpel/knife
Slide and coverslip
Bunsen burner/spirit burner
Microscope

Requirements for class

Acetic orcein stain in dropping bottles
1 molar hydrochloric acid in dropping bottles
45% ethanoic acid, in dropping bottles
Adjustable hotplate, if available
Beaker of detergent for the used slides
Growing onions: see above
Book illustrations, photos etc.



Safety Considerations

The Acetic orcein stain, ethanoic acid and the 1 molar hydrochloric acid all need to be treated with care, as they are corrosive. You may want to provide disposable gloves for the students.

Use of dropping bottles for the staining compounds makes the practical safer.

Care is needed with heating the watch glass: a spirit burner or adjustable hotplate is better, but a bunsen burner can be used if necessary. The students should just pass the watch glass through the roaring flame, turned down low.

Pathogens and Disease

2.1 Bacteria and viruses as pathogenic microorganisms

Bacteria

Practical work to include investigations into population growth of bacteria or yeast, involving sterile technique and the use of a haemocytometer.

An investigation of the population growth of yeast using a haemocytometer

Introduction

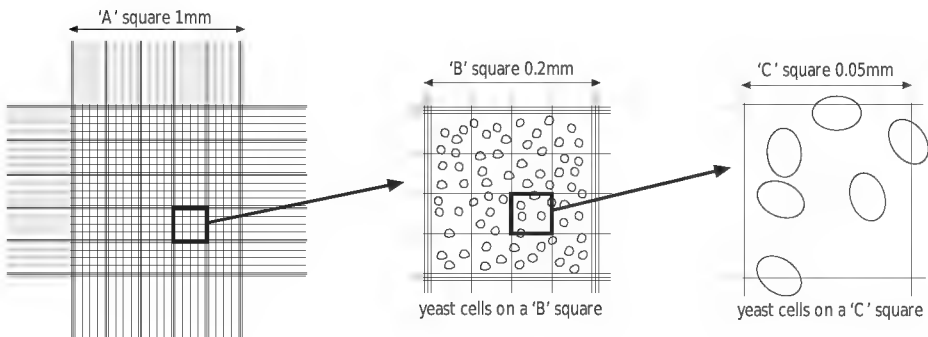
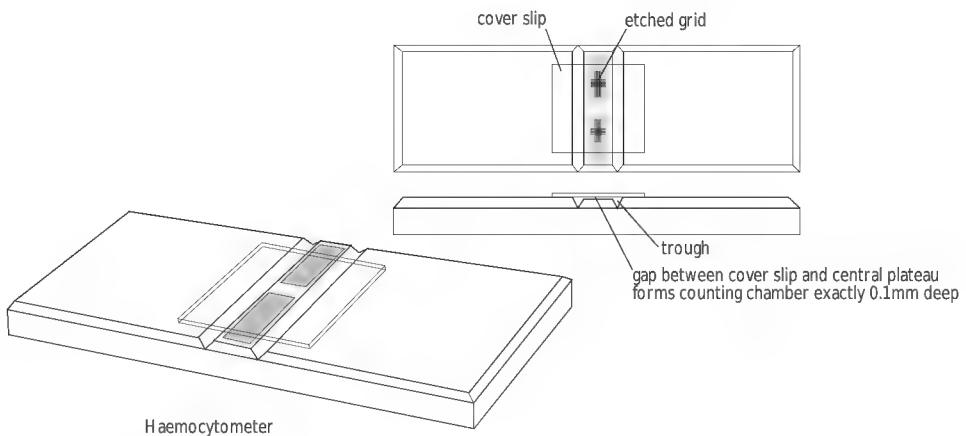
Population growth of micro-organisms is often measured using a haemocytometer, which is a type of microscope slide, originally devised to count blood cells. It has a grid marked on it, over which rests a known volume of liquid. By counting the number of cells visible against the grid, and multiplying by the known volume, an estimate for cell numbers can be obtained. (One advantage of this method if using bacteria is that results are obtained quite quickly, rather than having to inoculate agar plates and incubate them before counting colonies).

Method

- ▼ Collect a sterile 250cm³ conical flask containing malt extract broth, together with a test tube containing an active yeast culture.
- ▼ Using aseptic technique as demonstrated by the supervisor, add 1cm³ of yeast culture to the conical flask and swirl it carefully to mix. Put some cotton wool in the neck of the flask.
- ▼ Examine the haemocytometer slide by eye. There are usually two grids. You should just be able to see the markings in the centre of the two middle sections of the slide, either side of a central channel. These form grids of 1 mm², divided into smaller squares, as shown in the diagram. The central section of the slide is slightly lower and gives a precise depth when the coverslip is in position.

Figure: picture of haemocytometer slide and grid.

- ▼ Place a coverslip over the haemocytometer grids, pressing down firmly but carefully. If the coverslip is in the correct position, you should be able to see coloured rings under the coverslip, like the rings you see on a soap bubble: they are called “Newton’s Rings”. Using a sterile dropping pipette and aseptic technique, remove a small sample of the Yeast culture and run a drop under the coverslip of the haemocytometer (any excess solution runs off into the side channels).
- ▼ Examine the haemocytometer under the microscope. Adjust the microscope until you can see the yeast cells overlying the grid of the slide.
- ▼ Decide whether you are going to count cells which are budding as one cell or two. You also need to decide how you deal with cells that lie over the gridlines. The usual method is to count cells overlapping two sides of the grid, but not those overlapping the other two sides. (See diagram). Once you have decided these two points, stick to them.



continued

- ▼ Count the number of yeast cells in a selection of squares in the grid. To get an accurate estimate, you need to count about 150-200 yeast cells. Make a note of the number of squares you count, and the number of yeast cells per square to give a total. Use these figures to estimate the number of yeast cells per cm^3 of the culture. The volume of the whole grid is 0.1mm^3 , of a large square is 0.004mm^3 , and of a small square is 0.00025mm^3 . Use these figures as appropriate to calculate the number of yeast cells in the culture.
- ▼ Leave the yeast culture to incubate at $20\text{-}25^\circ\text{C}$ for 4-7 days. Count the number of cells, using the haemocytometer, at least once per day, making a note of the exact time when the culture was sampled. Remember to swirl the flask before sampling, to mix the cells evenly.
- ▼ Plot the results on ordinary graph paper, as cell numbers against time. You also need to make a plot of Log_{10} cell numbers against time. You can do this either by calculating the log_{10} figures from the data, or by using 'semi-log' graph paper, which has the lines already marked as a logarithmic scale.
- ▼ Compare the two graphs in your account.

Teaching Notes

This practical works well, but it might be useful for the students to try out using a haemocytometer first. You may also need to check their calculations the first time. If you want the students to use semi-log graph paper, it is probably best to get 6- or 7-cycle paper.

The students need to be introduced to basic aseptic technique, in particular flaming the top of the flask, and using a pipette aseptically. Cultures that become contaminated may be useful as object lessons!

The two graphs can be used to discuss the difference between an exponential plot and a semi-log plot, and to make the point that the usual “growth curve” plot is a semi-log graph. If the cultures grow well the students may be able to identify the various stages of a growth curve.

The practical may also provide the basis for further project work to examine differences in growth rates under different conditions, such as different media, or temperatures.

Requirements per student/group:

Haemocytometer

Sterile 1 cm³ pipette

Pipette filler

Several sterile dropping pipettes

Sterile 250 cm³ conical flask, containing 50 cm³ sterile malt extract broth

Active yeast culture

Safety Considerations

The students will need to be introduced to basic aseptic technique if they have not tried it before. However, by using a yeast such as *Saccharomyces cerevisiae*, the risks of any faults in technique are minimised.

If you are not familiar with standard microbiological aseptic techniques the following resources may be useful:

Microbiology: HMI Guide for Schools and F.E. (HMSO)

Practical Biotechnology: National Centre for Biotechnology Education, University of Reading.

‘An Introduction to Practical Microbiology’: a video from the Department of Science and Engineering, Manchester Metropolitan University.

Students may need reminding to handle the haemocytometers carefully, as they are rather expensive!

Safe use of the microscope.

Pathogens and Disease

3.4 Genetic information in cell division

Mitosis

Use of appropriate staining techniques in the study of mitosis in suitable plant material

Preparation of an onion root tip squash to show stages in mitosis

Introduction

An onion root tip is to be used to show the stages of mitosis in the dividing cells. The tip will need to be softened so the cells can be squashed to spread the chromosomes in one plane, and the chromosomes stained to see the stages of mitosis.

Method

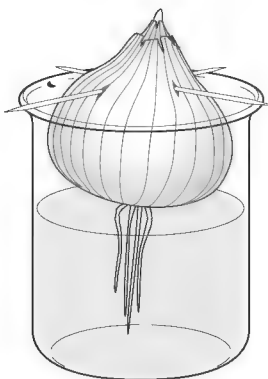
- ▼ Remove a root from a growing onion bulb. Carefully cut about 0.5cm from the tip of the root, making sure it is the tip end, not from the end near the onion. (It should be slightly pointed).
- ▼ Place the root tip in a watch glass. Add 9 drops of acetic orcein stain, and 1 drop of 1 molar hydrochloric acid. Heat the watch glass gently, using a hotplate or spirit burner, or by passing it carefully but quickly through the flame of a bunsen burner: DO NOT LET IT BOIL! Keep the watch glass hot but not boiling for 5 minutes: if you have to hold it, use forceps.
- ▼ Place the root tip on a clean slide, and add a drop of 45% ethanoic acid. Cut away all but the terminal 1mm. Cover with a clean coverslip.
- ▼ Squash the root tip gently using a pencil: hold the pencil vertically just above the coverslip, then let it fall through the fingers onto the coverslip. Repeat this 2-3 times. This should spread the cells out under the coverslip so they can be examined under the microscope.
- ▼ Examine the slide under the microscope, using the medium power (x10) objective lens, then high power (x40) objective lens. Identify the various stages of mitosis, and make annotated drawings of each stage.
- ▼ Record the final magnification of the drawing (eyepiece × objective × size difference)

Teaching Notes

Growing the onion roots needs to be started 6-7 days before the practical. It is best to use onion sets from a garden centre, or locally grown onions, as some onions bought from a supermarket have been treated to prevent sprouting.

Push two cocktail sticks through the onion at right angles: these will support the onion in the beaker, as shown in the diagram. Fill the beaker with water until it just covers the root area of the onion, and leave it in a cool place until the roots have sprouted. They do not have to be kept in the dark. Ideally, the roots need to be about 2 - 5 cm long.

If the onions are ready before the lesson is due, or if you wish to harvest onion roots and store them until needed, they can be treated with Carnoy's solution (6 parts absolute ethanol: 3 parts chloroform: 1 part glacial acetic acid) for 24 hours, then stored in 70% ethanol until needed.



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It can be useful to have a few prepared slides of root tip squashes or sections, in case the students have difficulty with the slide preparation. Appropriate photographs and / or diagrams will also be useful. In addition, it will be useful if the students can see video footage of mitosis. It is important to make it clear that mitosis is a continuous process, which is subdivided for convenience.

It might be possible for the students to estimate the relative length of each stage of mitosis, by counting how many cells they can see which are in each stage.

continued

Requirements per student

Watch glass
Forceps, scalpel/knife
Slide and coverslip
Bunsen burner/spirit burner
Microscope

Requirements for class

Acetic orcein stain in dropping bottles
1 molar hydrochloric acid in dropping bottles
45% ethanoic acid, in dropping bottles
Adjustable hotplate, if available
Beaker of detergent for the used slides
Growing onions: see below
Book illustrations, photos etc.

Safety Considerations

The Acetic orcein stain, ethanoic acid and the 1 molar hydrochloric acid all need to be treated with care, as they are corrosive. You may want to provide disposable gloves for the students.

Use of dropping bottles for the staining compounds makes the practical safer.

Care is needed with heating the watch glass: a spirit burner or adjustable hotplate is better, but a bunsen burner can be used if necessary. The students should just pass the watch glass through the roaring flame, turned down low.

Biology or Biology (Human)



**Examination Style
Question Papers**

Year Set 1

- 1 - Molecules, Cells & Systems**
- 2 - Applied Biology**
- 3 - Pathogens & Disease**

Complete with Assessment Grids & Mark Schemes

FELTHAM PRESS

Examination Style Question Papers

Name: _____

I - Molecules, Cells and Systems

Year Set I

Time allowed I hour 30 minutes

Instructions:

- Answer **all** the questions in the spaces provided.

Information

- Mark allocations are shown in brackets.
- The maximum mark for this paper is 75.
- just over half of the marks (43/75) will be for knowledge and understanding; just under half of the marks (32/75) will be for application of knowledge and understanding, analysis and evaluation;
- Quality of Written Communication will be assessed in answers to these questions.
- Scientific terminology should be used where appropriate.
- You may use a calculator.

Question I

The picture below shows part of an animal cell as seen under an electron microscope at a magnification of $\times 30\,000$, with all parts drawn accurately to scale.



- a) Identify structures **A** to **C**.

A _____

B _____

C _____ (3)

- b) Describe two ways in which a bacterial cell differs from the cell shown above.

i) _____

ii) _____

(2)

- c) Calculate the actual size of structure **C** as measured between the two points marked **X**. Show your workings.

(3)

- d) Name a structure in the drawing that is not visible even under the best light microscope.

(1)

Total 9

Question 2

Fick's law states that diffusion rate is proportional to:

surface area x difference in concentration/ thickness of exchange surface

a) Describe how each of the factors in the equation affects the rate of diffusion:

i) surface area

(1)

ii) difference in concentration

(1)

iii) thickness of exchange surface

(1)

b) What feature of a mitochondrion increases its surface area for diffusion?

(1)

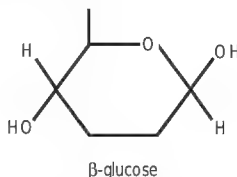
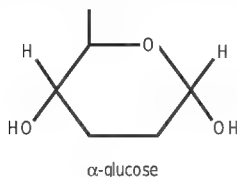
c) Explain how an increase in the aerobic activity of a cell increases the diffusion gradients of oxygen and carbon dioxide between the cytoplasm of the cell and the internal matrix of a mitochondrion.

(4)

Total 8

Question 3

- a) The structure of alpha glucose and beta glucose is shown below:



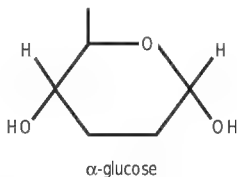
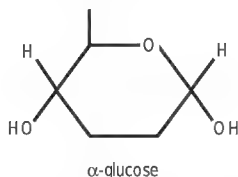
- i) The short-hand convention used in drawing organic molecules in this way omits the carbon atoms. Indicate on one of the diagrams above where the 6 carbon atoms of glucose are to be found

(1)

- ii) Describe how the two molecules shown differ in structure.

(1)

- b) Two alpha glucose molecules are shown below:



- i) Indicate on the diagram how the disaccharide maltose would be formed.

(1)

- ii) What type of reaction is this known as?

(1)

- iii) Name the chemical bond formed in this way.

(1)

- iv) Name and describe the type of reaction that would result in the two alpha glucose molecules being regained from the maltose molecule.

(2)

Total 7

Question 4

- a)** Give a definition of the term catalyst.

(1)

- b)** Name an enzyme and the reaction that it catalyses.

(1)

- c** What is meant by the term 'enzyme specificity'

(1)

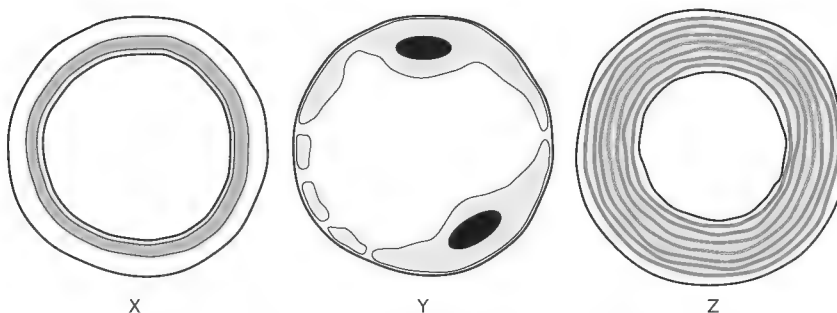
- d)** Enzymes are denatured by extremes of pH. Explain what is meant by the term 'denatured'

(2)

Total 5

Question 5

The three diagrams below are of cross (transverse) sections of an artery, vein and capillary, not drawn to scale.



- a) i) Identify the artery, vein and the capillary.

(1)

- ii) What is the diameter of an average capillary?

(1)

- b) Describe how the structure of the capillary is adapted to its function.

(3)

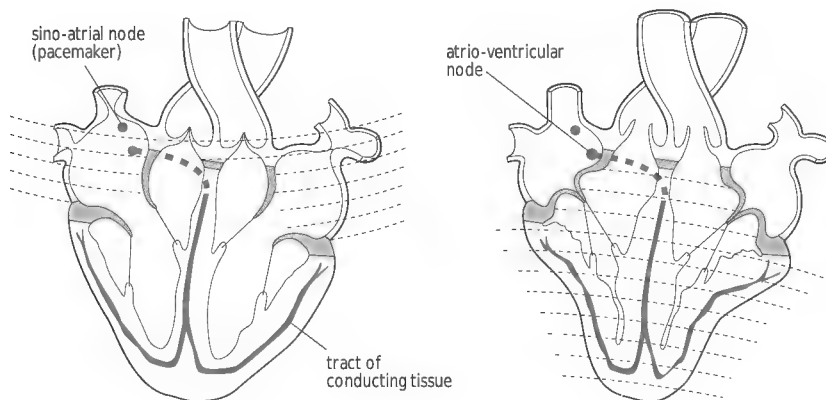
- c) Explain in terms of lung function, why it is necessary for the blood in the lung capillaries to be under a much lower pressure than that in the capillaries of the muscles.

(3)

Total 8

Question 6

The diagrams below show vertical sections of the heart at different stages of the cardiac cycle. The spread of electrical activity through the walls is indicated by the radiating curved lines.



- a) Draw arrows on the diagram above to show the directions in which electrical activity spreads through the walls of the heart. (1)

- b) Relate this pattern of spread of electrical activity, and the resultant contractions of the atria and ventricles in the cardiac cycle, to the direction of blood flow through the heart. (3)

- c) Explain what causes the heart valves to open and close during the cardiac cycle. (2)

Total 6

Question 7.

- a)** Ventilation of the lungs is by tidal flow in and out of a single air tube the trachea. Describe the main disadvantage of this method of ventilating a respiratory system?

(2)

- b)** The surfaces of the alveoli are covered in a layer of water and mucus.

- i)** Describe how this layer of water and mucus is formed.

(2)

- ii)** Explain whether the existence of this layer of water and mucus increases or decreases the rate of gaseous exchange between the blood and the alveolar air.

(2)

Total 6

Question 8

Read the following passage

Neither the light microscope nor the electron microscope reveals much of the structure of the cell membrane. Models of membrane structure are constructed on the basis of the membrane's behaviour under certain conditions; in other words, it is impossible to separate structure and function since our knowledge of the structure derives from our knowledge of function.

Early investigations were based on studies of the rate of penetration of various materials into the cell, e.g. urea, sugars, alcohols, etc. It was found that fat-soluble substances penetrated more easily than others. Lipids were extracted from red blood cell membranes, and the surface area that this lipid would have if it formed a layer one molecule thick was measured and found to be twice the surface area of the red blood cells.

Further work indicated that 'smaller' molecules penetrated the membrane faster than would have been expected on the basis of their fat solubility alone. It was then shown that the surface tension of the membrane was lower than expected of an exposed lipid layer, they therefore postulated that protein was adsorbed on to the membrane surface. It was suggested that all membranes showed this common structure. However, the wide variety of functions shown by various membranes seemed to argue against this. More work on the red blood corpuscle membrane indicated that many protein molecules span the membrane from its internal to its external surface. As techniques of investigation increase, so the apparent structure of the membrane becomes more complex; an excellent example of the way in which our understanding is determined by available techniques.

Use information from the passage and your knowledge to answer the following questions

- a)** Why do neither the light microscope nor the electron microscope reveal much of the structure of the cell membrane?

(2)

- b)** What does the fact that fat soluble substances enter the cell more rapidly than non-fat soluble substances suggest about the nature of the membrane?

(1)

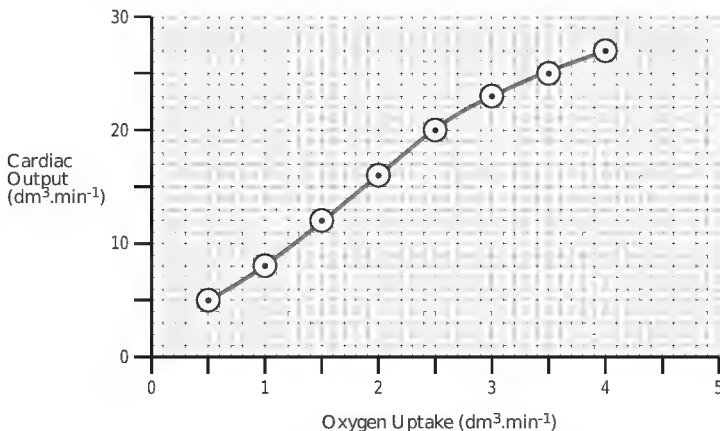
- c)** What does the finding that the surface area of lipid extracted from the red cell membranes would have formed a layer one molecule thick with twice the surface area of the red blood cells tell us about the arrangement of lipids in the red blood cell membrane?

(1)

Question continued...

Question 9

Study the graph below of cardiac output plotted against oxygen uptake at increasing sub-maximal work rates on a cycle ergometer and then answer the following questions.



- a) i) Briefly describe the relationship between oxygen uptake and cardiac output shown in the graph.

(2)

- ii) Explain in terms of heart and lung function why they are related in this way.

(4)

- b) Why does an aerobically fit individual generally have a lower resting heart rate than an aerobically unfit individual of the same size and sex?

(8)

Total 14

Assessment Grid

Unit I - Molecules, Cells and Systems Year Set I

Specification Section	Question Number									Total
	1	2	3	4	5	6	7	8	9	
10.1 Cell Structure	3									3
10.2 Ultrastructure	6							12		18
10.3 Membrane & Cell Transport		8								8
10.4 Biological Molecules			7							7
10.5 Enzymes				5						5
10.6 Tissue and Organs					5					5
10.7 Blood System					3		6			9
10.8 Heart						6			14	20
Total										75

Assessment Objective	Question Number									Total
	1	2	3	4	5	6	7	8	9	
AO1										
Knowledge with Understanding	6	4	5	5	5	1	4	6	6	42
AO2										
Application/Analysis/Evaluation	3	4	2		3	5	2	6	8	33
Total										75

Mark Schemes for Question Paper

I - Molecules, Cells and Systems Year Set I

Instructions

; = 1 mark / = alternative response

Question I

The drawing shows part of a cell as seen under an electron microscope at a magnification of $\times 30\,000$, with all parts drawn accurately to scale.

- a) Identify structures A to C.
A nucleus;
B mitochondrion;
C rough or granular endoplasmic reticulum; (3)
- b) Describe two ways in which a bacterial cell differs from the cell shown above.
i) & ii) any two from
bacterium cell has no mitochondrion;
no nucleus;
no endoplasmic reticulum; (2)
- c) Calculate the actual size of structure C as measured between the two points marked X. Show your workings.
distance between points x-x = 30 mm;
magnification = $\times 30\,000$
therefore actual size = 30 divided by 30 000;
= 0.001 mm / 1.0 mm; (3)
- d) Name a structure in the drawing that is not visible even under the best light microscope?
ribosomes; (1)

Total 9

Question 2

Fick's law states that diffusion rate is proportional to:

surface area x difference in concentration/ thickness of exchange surface

a) Describe how each of the factors in the equation affects the rate of diffusion:

i) surface area

the larger the surface area the faster the diffusion rate;

(1)

ii) difference in concentration

the larger the difference in concentration the faster the diffusion rate;

(1)

iii) thickness of exchange surface

the thicker the exchange surface the slower the diffusion rate/ vice versa;

(1)

b) What feature of a mitochondrion increases its surface area for diffusion?

the folding(s) of the internal membrane / cristae;

(1)

c) Explain how an increase in the aerobic activity of a cell increases the diffusion gradients of oxygen and carbon dioxide between the cytoplasm of the cell and the internal matrix of the mitochondrion.

as the activity of a cell increases more ATP required;

more oxygen used;

and more carbon dioxide produced;

in aerobic respiration in the internal matrix of the mitochondrion;

difference between the levels of these two gases in the matrix of the mitochondrion and the cytoplasm of the cell increased;

(4)

Total 8

Question 3

- a) The structure of alpha glucose and beta glucose are shown below:
- i) The short-hand convention used in drawing organic molecules in this way omits the carbon atoms. Indicate on one of the diagrams above where the 6 carbon atoms of glucose are to be found.
all 6 carbons accurately marked; (1)
- ii) Describe how the two molecules shown differ in structure.
positions of OH and H groups on carbon 1 reversed; (1)
- b) Two alpha glucose molecules are shown below:
- i) Indicate on the diagram how the disaccharide maltose would be formed.
removal of elements of water correctly indicated; (1)
- ii) What type of reaction is this known as?
condensation reaction; (1)
- iii) Name the chemical bond formed in this way.
glycosidic bond (1)
- iv) Name and describe the type of reaction that would result in the two alpha glucose molecules being regained from the maltose molecule.
hydrolysis reaction;
elements of water added;
across the glycosidic bond; (2)

Total 7

Question 4

- a) Give a definition of the term catalyst.
a substance which speeds up the rate of a reaction;
without itself being used up;
both needed for the mark; (1)
- b) Name an enzyme and the reaction that it catalyses.
both needed for the mark; (1)
- c) What is meant by the term 'enzyme specificity'
an enzyme will only catalyse a specific reaction or type of reaction; (1)
- iv) Enzymes are denatured by extremes of pH. Explain what is meant by the term 'denatured'
rendered irreversibly inactive;
as a result of permanent disruption of their 3 D structure / active site; (2)

Total 5

Question 5

The three diagrams below are of cross (transverse) sections of an artery, vein and capillary, not drawn to scale

- a) i) Identify the artery, vein and the capillary.

X = vein; Y = capillary; Z = artery;

(1)

- ii) What is the diameter of an average capillary?

6 - 8 μm

(1)

- b) Describe how the structure of the capillary is adapted to its function.

thin walled / wall one cell thick;

some also have pores in the wall / fenestrated;

diameter same as that of the red blood cells;

(3)

- c) Explain in terms of lung function, why it is necessary for the blood in the lung capillaries to be under a much lower pressure than the blood in the capillaries of the muscles.

capillaries of lungs close to / next to air in air sacs (alveoli);

if blood pressure same as that in muscle capillaries much tissue fluid exuded;

alveoli would fill with tissue fluid;

reducing surface area for gas exchange by diffusion;

increasing diffusion distance for gas exchange by diffusion;

(3)

Total 8

Question 6

The diagrams below show vertical sections of the heart at different stages of the cardiac cycle. The spread of electrical activity spreads through the walls is indicated by the radiating curved lines.

- a) By means of arrows on the diagram above show the directions in which electrical activity through the walls of the heart.
arrows downwards and across in atria, and upwards in ventricles, possibly down septum; (1)
- b) Relate this pattern of spread of electrical activity to the direction of the flow of blood as a consequence of the resultant contractions of the atria and ventricles in the cardiac cycle.
stimulus radiating downwards in atria results in downward contraction of atria;
forcing blood downwards into the ventricles;
stimulus radiating upwards in ventricles results in upward contraction of ventricles;
forcing blood upwards into pulmonary arteries and main aorta; (3)
- c) Explain what causes the heart valves to open and shut during the cardiac cycle.
heart valves are passive structures incapable of initiating movement;
pressure of blood forces valves open or shut;
dependent upon their one-way arrangement; (2)

Total 6

Question 7.

- a) Ventilation of the lungs is by tidal flow in and out of a single air tube the trachea. Describe the main disadvantage of this method of ventilating of a respiratory system?
- inefficient flushing of air through the system;
intermittent (stop-start) flow of air into system;
a volume of stationary air always present in the system;
diffusion gradients for gaseous exchange less steep; (2)
- b) The surfaces of the alveoli are covered in a layer of water and mucus.
- i) Describe how this layer of water and mucus is formed.
- water film an unavoidable consequence of a water potential gradient;
between the alveolar cells and the air in the alveoli;
mucous secreted to reduce evaporative losses (2)
- ii) Explain whether the existence of this layer of water and mucus increases or decreases the rate of gaseous exchange between the blood and the alveolar air.
- decrease;
the layer of water and mucus increases the diffusion distance for gaseous exchange;
oxygen diffuses faster as a gas than in solution; (2)

Total 6

Question 8

Use information from the passage and your knowledge to answer the following questions

- a) Why do neither the light microscope nor the electron microscope reveal much of the structure of the cell membrane?
the resolving power of the LM is insufficient;
the resolving power of the EM is insufficient;
preparation techniques for the EM are too drastic to leave the membrane unaltered; (2)
- b) What does the fact that fat soluble substances enter the cell more rapidly than non-fat soluble substances suggest about the nature of the membrane?
the membrane is fatty in nature; (1)
- c) What does the finding that the surface area of lipid extracted from the red cell membranes would have formed a layer one molecule thick with twice the surface area of the red blood cells tell us about the arrangement of lipids in the red blood cell membrane?
suggests that there were two layers of lipid in the membrane;
arranged as a bimolecular lipid membrane; (1)
- d) What does the fact that smaller molecules penetrated the membrane faster than would have been expected on the basis of their fat solubility alone suggest about membrane structure?
that the membrane is porous;
allowing smaller molecules to penetrate faster through pores of a certain diameter;
independent of their fat solubility therefore pores are 'aqueous'; (2)
- (e) Describe in words the structure of the modern fluid mosaic model of the cell surface membrane.
two layers of phospho-lipids (bilayer);
hydrophobic poles together, hydrophilic poles 'outwards';
stabilised by cholesterol;
surface proteins;
glycoproteins;
glycolipids;
embedded proteins;
'pore' proteins; (6)

Total 12

Question 9.

Study the graph below of cardiac output plotted against oxygen uptake at increasing sub-maximal work rates on a cycle ergometer and then answer the following questions.

- a) i) Briefly describe the relationship between oxygen uptake and cardiac output shown in the graph.
as one increases so does the other;
proportionally / linearly;

(2)

- ii) Explain in terms of heart and lung function why they are related in this way.

oxygen uptake in the lungs is by diffusion of oxygen into blood from alveoli;

blood supply is via the pulmonary arteries from the heart;

blood leaving lungs in pulmonary veins is always saturated with oxygen at sea level;

increased uptake of oxygen is achieved with increased flow in pulmonary arteries;

supplied by increased cardiac output;

(4)

- b) Why does an aerobically fit individual generally have a lower resting heart rate than an aerobically unfit individual of the same size and sex?

heart rate related to demand by tissues for oxygen;

cardiac output related to demand by tissues for oxygen;

at rest the demand for oxygen of the two individuals is the same;

the aerobically fitter individual will have a larger stroke volume;

as a result of increased contractility of ventricle walls;

and possibly increased volume of ventricles (athlete's heart);

cardiac output is a function of heart rate and stroke volume;

therefore same cardiac output achieved with a slower resting heart rate;

(8)

Total 14

Examination Style Question Papers

Name: _____

2 - Applied Biology Year Set I

Time allowed | hour 30 minutes

Instructions:

- Answer **all** the questions in the spaces provided.

Information

- Mark allocations are shown in brackets.
- The maximum mark for this paper is 75.
- just over half of the marks (43/75) will be for knowledge and understanding; just under half of the marks (32/75) will be for application of knowledge and understanding, analysis and evaluation
- Quality of Written Communication will be assessed in answers to these questions.
- Scientific terminology should be used where appropriate.
- You may use a calculator.

Question 1

Fructose is produced from glucose on an industrial scale by the use of immobilised enzymes.

- a)** Explain what is meant by the term 'immobilised enzymes'

(1)

- b)** State three advantages of using immobilised enzymes.

(3)

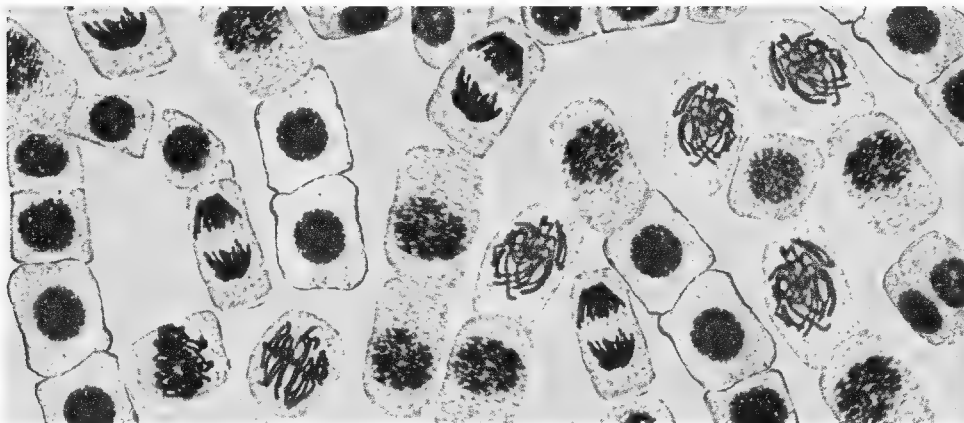
- c)** Glucose syrup is poured slowly through columns of immobilised glucose isomerase enzyme which converts glucose to fructose. High fructose syrup (42% fructose and 54% glucose) is produced which has the same 'sweetness' as sucrose. Explain why this process is important to economies with easy access to starch, but not to sugar cane or sugar beet.

(3)

Total 7

Question 2

Study the illustration below of stained cells of a squashed plant root tip, showing various stages of mitosis, and answer the following questions.



a) i) Indicate with an X on the illustration a cell which is showing the separation of chromatids (1)

ii) Indicate with a Y on the illustration a cell which is showing the chromosomes lining up on the equator of the nuclear spindle. (1)

b) Explain why the specimen was **squashed** and not sectioned.

(5)

c) Explain why plant root tips are good source of cells undergoing mitosis.

(2)

Total 9

Question 3

Read the passage below that sets out some of the evidence that DNA is the genetic material, and answer the following questions.

The bacterium *Diplococcus pneumoniae* has a virulent strain with smooth capsules (S) and a non-virulent strain with rough capsules (R). It was found that, if a mixture of heat-killed (S) and living (R) was injected into mice, living (S) could be isolated, and that this transformation was stable and inherited. Later experiments showed that the only substance in the bacteria that could bring about this transformation by passing from the heat-killed (S) into the living (R) types was a substance known as deoxyribonucleic acid (DNA). Modification of the *Diplococcus pneumoniae* capsules is very common, for example, if it is grown on a sucrose-containing medium the capsules are smooth, and if on a glucose-containing medium the capsules are rough. However, the transformation by DNA is both stable and inherited, indicating the role of DNA as the genetic material. Other evidence was gained from work on viruses. These consist basically of a thread of DNA protected inside a protein coat, and some viruses infect cells by attaching to the cell surface and injecting their DNA into the cytoplasm of the host, leaving a 'protein ghost' outside the cell. The injected DNA then directs the host cytoplasm to construct complete viruses which are liberated with the destruction of the host cell. It was the investigation of this process of virus infection and reproduction that further demonstrated the role of DNA as the genetic material.

- a)** Explain why the use of heat-killed strain (S) eliminates protein from being the genetic material. (line 3)

(2)

- b)** Explain why the work with viruses also eliminates protein from being the genetic material. (lines 11-15)

(2)

- c)** Name the form in which DNA is regularly exchanged between bacteria.

(1)

- d)** Briefly explain in your own words why genetic transformation demonstrates that DNA is the genetic material. (lines 1-4 & 8-10)

(3)

Total 8

Question 4

a) Briefly describe the reactions catalysed by the following enzymes.

i) reverse transcriptase

(2)

ii) restriction endonuclease

(2)

iii) ligase

(2)

b) In the context of gene technology explain the meaning of the term 'vector'.

(2)

c) Explain the principles behind the use of 'genetic markers' in gene technology.

(4)

Total 12

Question 5

Study the the results below of some tests for the ABO blood groups and answer the following questions.

Antibody that the blood sample was tested against

Blood Sample	anti-A	anti-B	none (control)
X	agglutinated	no reaction	no reaction
Y	no reaction	agglutinated	no reaction
Z	agglutinated	agglutinated	no reaction

- a) Identify the blood group of each sample tested, and explain the reason for each identification.

i) **X**

(2)

ii) **Y**

(2)

iii) **Z**

(2)

- b) Explain why blood of group O is referred to as 'universal donor' blood.

(3)

Total 9

Question 6

a) Define the following terms:

i) leaching;

(2)

ii) eutrophication.

(2)

b) Describe the relationship between the two terms with reference to nitrate fertilisers.

(3)

Total 7

Question 7

a) Give a definition for each of the following terms:

i) diploid chromosome number;

(1)

ii) haploid chromosome number;

(1)

b) Briefly explain gamete formation and fertilisation using the terms diploid chromosome number and haploid chromosome number.

(3)

Total 5

Question 8

Although Maize is now also grown in temperate climates, it is originally and essentially a tropical plant. In addition to the C3 method of photosynthesis found in all plants, Maize has a specialised C4 method of photosynthesis which adapts it to tropical conditions. The disadvantage of C3 photosynthesis is that at high light intensities there is light stimulated photo-respiration.

- a) Explain how light stimulated photo-respiration limits the efficiency of C3 photosynthesis.

(3)

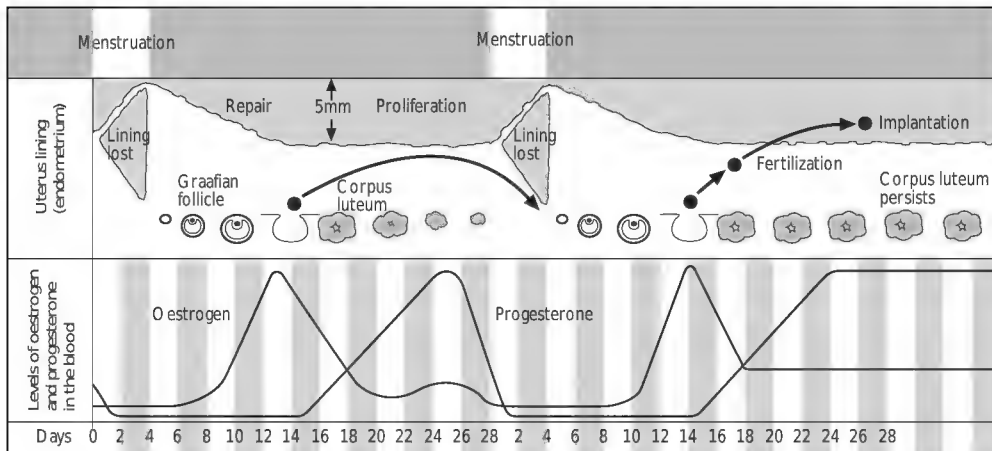
- b) Describe the main features of C4 photosynthesis.

(7)

Total 10

Question 9

Study the diagram below of the ovarian (menstrual) cycle in the human female and answer the following questions.



- a) i) What event in the ovary coincides with the peak of oestrogen levels in the blood?

(1)

- ii) What event results in the maintenance of high levels of progesterone in the blood?

(1)

- b) Describe how contraceptive pills act to control human fertility.

(6)

Total 8

Assessment Grid

2 - Applied Biology Year Set 1

Specification Section	Question Number									Total
	1	2	3	4	5	6	7	8	9	
10.1 Enzymes	7									7
10.2 Mitosis		9					5			14
10.3 DNA			8							8
10.4 Gene technology				12						12
10.5 Forensic science					9					9
10.6 Plant growth						7		10		17
10.7 Biotechnology/Reproduction									8	8
Total										75

Assessment Objective	Question Number									Total
	1	2	3	4	5	6	7	8	9	
AO1										
Knowledge with Understanding	4	6	1	8	6	7	2	7	2	43
AO2										
Application/Analysis/Evaluation	3	3	7	4	3		3	3	6	32
Total										75

Mark Schemes for Question Paper

2 - Applied Biology Year Set I

Instructions

; = 1 mark / = altern

Question I

Fructose is produced from glucose on an industrial scale by the use of immobilised enzymes.

- a) Explain what is meant by the term 'immobilised enzymes'
conversion of water soluble enzyme into a water insoluble product;
which retains its catalytic activity; (1)
- b) State three advantages of immobilised enzymes.
immobilised enzymes easily separated from reactants and products;
easy collection of enzyme-free product;
thus giving higher degree of control;
they are also more stable; (3)
- c) Glucose syrup is poured slowly through columns of immobilised glucose isomerase enzyme which converts glucose to fructose. High fructose syrup (42% fructose and 54% glucose) is produced which has the same 'sweetness' as sucrose. Explain why this process is important to economies with easy access to starch, but not to sugar cane or sugar beet.
sugar cane and sugar beet are sources of sucrose;
starch can be converted to glucose;
sucrose sweeter than glucose;
by converting a fraction to fructose can achieve same sweetness as with sucrose; (3)

Total 7

Question 2

Study the illustration below of a stained slide of a squashed plant root tip, showing various stages of mitosis, and answer the following questions.

- a) i) Indicate with an X on the illustration a cell which is showing the separation of chromatids.
nucleus correctly identified (1)
- ii) Indicate on the illustration a nucleus which is showing the chromosomes lining up on the equator of the nuclear spindle.
nucleus correctly identified (1)
- b) Explain why the specimen was **squashed** and not sectioned.
squashing separates out the individual chromosomes;
in one plane;
so all can be seen;
sectioning would only catch parts of chromosomes; (5)
- c) Explain why plant root tips are good source of cells undergoing mitosis.
growing regions;
apical meristem just behind tip;
produce nuclei by mitosis in all directions; (2)

Total 9

Question 3

Read the passage below that sets out some of the evidence that DNA is the genetic material, and answer the following questions.

- a) Explain why the use of heat-killed strain (S) eliminates protein from being the genetic material. (line 3)
proteins are denatured by heat;
but genetic material still functioning; (2)
- b) Explain why the work with viruses also eliminates protein from being the genetic material. (line 11-15)
only the nucleic acid enters the host cell;
protein coat remains on surface;
as protein 'ghost'; (2)
- c) Name the form in which DNA is regularly exchanged between bacteria.
plasmids / circular DNA in cytoplasm; (1)
- d) Briefly explain in your own words why genetic transformation demonstrates that DNA is the genetic material. (lines 1-4 & 8-10)
genetic transformation is stable and inherited;
DNA is the only material that could have passed;
from heat killed S to living R;
DNA converts living R to living S; (3)

Total 8

Question 4

- a) Briefly describe the reactions catalysed by the following enzymes.
- i) reverse transcriptase
formation of DNA;
from mRNA; (2)
 - ii) restriction endonuclease
hydrolysis at specific points along nucleic acid;
'cutting' it into shorter lengths; (2)
 - iii) ligase
rejoins;
'cut' ends of nucleic acids; (2)
- b) In the context of gene technology explain the meaning of the term 'vector'.
carries the engineered gene;
into the host cell; (2)
- c) Explain the principle behind the use of 'genetic markers' in gene technology.
engineered genes recombined into host DNA;
not always easily located / have no easily demonstrated phenotypic effect;
therefore are linked to a 'marker' gene which does;
if genetic marker is detected then linked engineered gene also present;
e.g. antibiotic resistance in bacteria; (4)

Total 12

Question 5

Study the illustration of the results below of some tests for the ABO blood groups and answer the following questions.

Antibody that the blood sample was tested against

Blood Sample	anti-A	anti-B	none (control)
X	agglutinated	no reaction	no reaction
Y	no reaction	agglutinated	no reaction
Z	agglutinated	agglutinated	no reaction

a) Identify the blood groups of the tests, and explain the reasons for each identification.

i) X

blood group A

agglutinated by anti-A antibody

(2)

ii) Y

blood group B

agglutinated by anti-B antibody

(2)

iii) Z

blood group AB

agglutinated by both anti-A and anti-B antibodies

(2)

b) Explain why blood of group O blood is referred to as 'universal donor' blood.

has no A/B antigens on surface of red blood cells;

therefore cannot be attacked by antibodies of any recipient group;

under conditions of transfusion only recipient antibodies significant;

(3)

Total 9

Question 6

a) Define the following terms:

i) leaching;

draining or washing out of soluble substances;
from soil and/or rocks into bodies of water;

(2)

ii) eutrophication.

saturation of bodies of water;
with nutrients/nitrates;

(2)

b) Describe the relationship between the two terms with reference to nitrate fertilisers.

excess nitrate from fertilisers;
not strongly held by soil particles;
therefore easily leached out by rainfall;
causing eutrophication of bodies of water;

(3)

Total 7

Question 7

- a) Give a definition for each of the following terms:
- i) diploid chromosome number;
two sets of chromosomes;
chromosomes occurring in homologous pairs; (1)
 - ii) haploid chromosome number;
one set of chromosomes / one of each pair of homologous chromosomes; (1)
- b) Briefly explain gamete formation and fertilisation using the terms diploid chromosome number and haploid chromosome number.
diploid organisms;
produce haploid gametes;
fertilisation restores diploid number of chromosomes; (3)

Total 5

Question 8

Although Maize is now also grown in temperate climates, it is originally and essentially a tropical plant. In addition to the C3 method of photosynthesis found in all plants, Maize has a specialised C4 method of photosynthesis which adapts it to tropical conditions. The disadvantage of C3 photosynthesis is that at high light intensities there is a light stimulated photo-respiration.

- a) Explain how light stimulated photo-respiration limits the efficiency of C3 photosynthesis
- at low light intensity light is limiting factor;
increase light intensity increase rate of photosynthesis;
up to an optimum level;
past optimum products lost due to respiration; (3)
- b) Describe the main features of C4 photosynthesis
- carbon dioxide does not enter the chloroplasts of the leaf mesophyll cells;
but is bound to a substance in the cytoplasm;
the resulting compound, is immediately converted to other substances;
then actively transported into specialised **Kranz cells**;
carbon dioxide is released into the chloroplasts of the Kranz cells;
where it is combined into organic compounds in the the normal way.
- C4 photosynthesis separates the process of oxygen release (the light dependent stage) from that of carbon dioxide assimilation (the non-light dependent stage);
the former occurring in the mesophyll cells the latter in the Kranz cells;
the main enzyme involved does not 'pick up' oxygen thus eliminating photorespiration; (7)

Total 10

Question 9

Study the diagram of the ovarian (menstrual) cycle in the human female and answer the following questions.

- a) i) What event in the ovary coincides with the peak of oestrogen levels in the blood?
ovulation; (1)
- ii) What event results in the maintenance of high levels in the blood of progesterone?
fertilisation / maintenance of the corpus luteum; (1)
- b) Describe how contraceptive pills act to control human fertility.
combined pill - contains oestrogen and progesterone or similar molecules;
these act to inhibit the production of FSH and LH;
therefore prevent the further development of Graafian Follicles and ovulation;
mini-pill - progesterone or equivalent only;
thought to act by preventing sperm reaching the secondary oocyte;
by altering the mucus of the vagina/cervix;
or by preventing the implantation of the blastocyst in the endometrium;
morning-after pill; (6)

Total 8

Examination Style Question Papers

Name: _____

3 - Pathogens and Disease Year Set I

Time allowed | hour 30 minutes

Instructions:

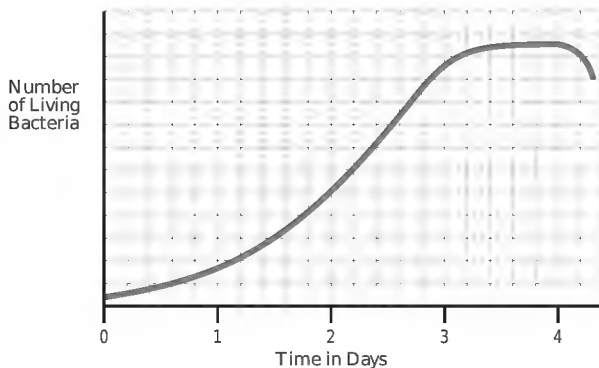
- Answer **all** the questions in the spaces provided.

Information

- Mark allocations are shown in brackets.
- The maximum mark for this paper is 75.
- just over half of the marks (43/75) will be for knowledge and understanding; just under half of the marks (32/75) will be for application of knowledge and understanding, analysis and evaluation;
- Quality of Written Communication will be assessed in answers to these questions.
- Scientific terminology should be used where appropriate.
- You may use a calculator.

Question 1

Study the sigmoid growth curve of a bacterial population incubated at 20°C below and answer the questions that follow



- a) Mark and label the following phases on the curve of the graph: decline phase, log phase, lag phase, and stationary phase. (2)

- b) Describe what is occurring in the bacterial population during the log phase.

_____ (2)

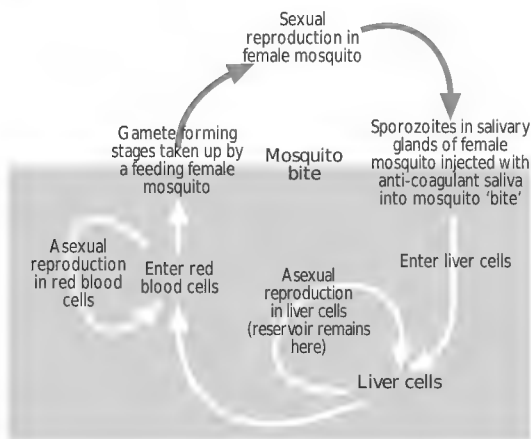
- c) Describe what is occurring in the bacterial culture during the decline phase.

_____ (2)

Total 6

Question 2

A simplified diagram of the malarial parasite (*Plasmodium*) life cycle is shown below.



- a) Explain why the female mosquito injects saliva into the individual on which she is going to feed.
- _____
- _____ (1)
- b) A parasite must be able to survive the hostile environment within the host. Describe the way in which the blood stream could be considered:
- i) an ideal environment for the malarial parasite;
- _____
- _____ (1)
- ii) a hostile environment for the malarial parasite;
- _____
- _____ (1)
- c) Describe those features of the life cycle of the malarial parasite that could be considered to be associated with the infection of a new host.
- _____
- _____
- _____
- _____ (3)

Total 6

Question 3

Read the following passage, and using information from the passage and your own knowledge answer the questions that follow.

B-lymphocytes form memory cells after meeting an antigen, e.g. a pathogen, in the body. These memory cells allow the body to 'remember' antigens it has met before and produce a much faster immune response to them the second time around.

When they do meet a familiar antigen, B-lymphocyte memory cells divide and form new antibody producing plasma cells. Some memory cells always remain so that the body can respond in this way to any number of future infections with the same antigen. The person is said to be resistant or immune to that disease. The immunity to some diseases lasts a life time, but some illnesses can be suffered more than once.

Larger amounts of antibodies are produced more rapidly by B-lymphocytes in the secondary response than in the first (or primary) response to a pathogen.

a) Describe what is meant by terms:

i) antigen

(1)

ii) antibody

(1)

iii) pathogen

(1)

b) Describe the relationship between pathogens and antigens.

(2)

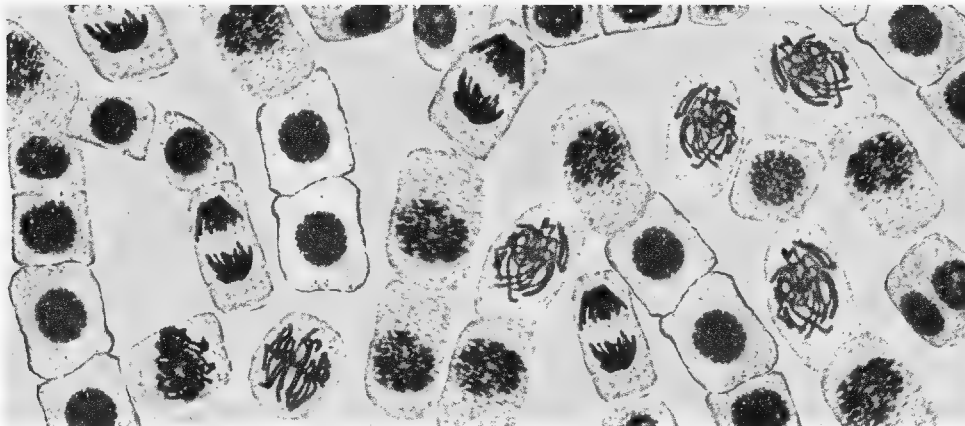
c) Explain how the immunity to some diseases lasts a life time, but some illnesses can be suffered more than once

(2)

Total 7

Question 4

Study the illustration below of stained cells of a squashed plant root tip, showing various stages of mitosis, and answer the following questions.



a) i) Indicate with an X on the illustration a cell which is showing the separation of chromatids (1)

ii) Indicate with a Y on the illustration a cell which is showing the chromosomes lining up on the equator of the nuclear spindle. (1)

b) Explain why the specimen was **squashed** and not sectioned.

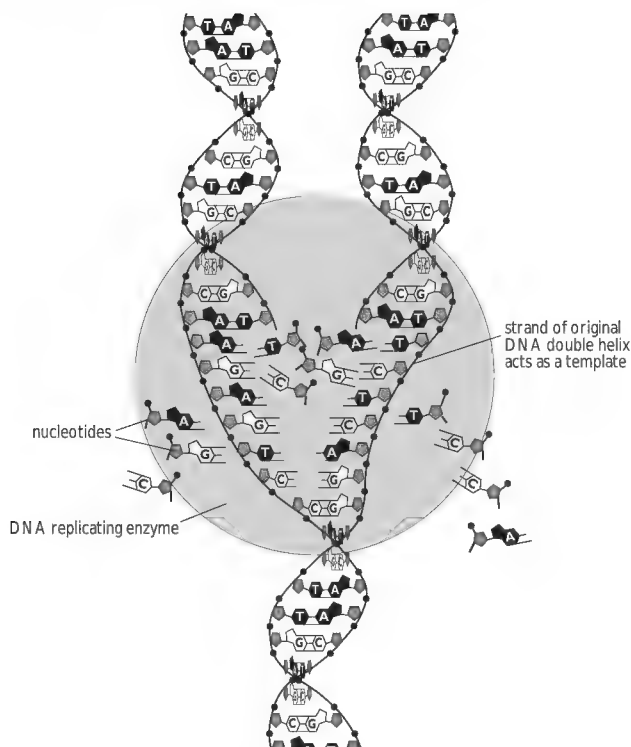
(2)

c) Explain why plant root tips are good source of cells undergoing mitosis.

(1)

Total 5

Question 5



Study the diagram above of replicating DNA then answer the questions that follow.

- a) On the diagram:
- i) label the two new strands of DNA; (1)
 - ii) mark with an X a pair of complementary bases; (1)
 - iii) indicate with an arrow on the diagram the direction in which the new strands of DNA are being formed; (1)
- b) With reference to the diagram:
describe what is meant by semi-conservative replication;
- _____
- _____
- _____ (3)
- c) Name an enzyme involved in catalysing the chemical reactions of DNA replication
- _____ (1)

Total 7

Question 6

The table below summarises the effects of two antibiotics on genetically modified (engineered) bacteria, which had been exposed to plasmids containing either the two genes for resistance to the two antibiotics in the table, or the gene for resistance to one antibiotic, as well as the gene for human insulin.

Bacteria	Ampicillin	Tetracycline
A	No	No
B	Yes	Yes
C	Yes	No

- a) i) Describe the nature of plasmids.

(1)

- ii) Describe and explain the treatment to which bacteria can be exposed that encourages the uptake of plasmids.

(2)

- iii) From the results shown in the table describe the state of bacteria A, B, and C in terms of which plasmids they have taken up.

A

B

C

(1)

- b) Given the results in the table, briefly describe how bacteria carrying the gene for human insulin could be isolated from a mixed culture.

(3)

Total 7

Question 7

- a) i) The majority (70%) of white cells in the blood are phagocytic granulocytes. Describe the process of phagocytosis.

(3)

- ii) Granulocytes only survive in the blood and tissue fluids for up to about 7 days. Suggest a reason why being phagocytic might contribute to this relatively short existence span.

(1)

- b) Explain how the granulocytes can concentrate around points of infection outside of the bloodstream.

(2)

Total 6

Question 8

- a) Name two characteristics of enzymes that enable them to be used as analytical reagents.

(2)

- b) The enzyme glucose oxidase catalyses the conversion of glucose to gluconic acid. Glucose oxidase, immobilised in a semi-conducting silicon chip, can give a measure of the level of blood glucose by the generation of a small electric current which can be converted to a liquid crystal display.

- i) Name a condition in which the accurate measurement of blood glucose is vital.

(1)

- ii) Explain how the conversion that glucose oxidase catalyses can result in an accurate measure of blood glucose level.

(3)

Total 6

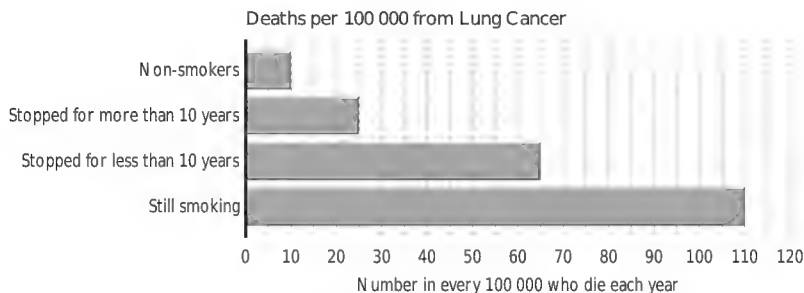
Question 9

Study the data presented below on smoking and lung cancer, and then answer the questions that follow.

Between 30 000 to 50 000 die from lung cancer each year in the UK.

Between 33% - 35% of smokers die from lung cancer.

Lung cancer accounts for one in seven deaths in males between 55-64 years of age.



- a) Suggest an explanation for why there is such a high death rate from lung cancer in males between 55-64 years of age.

(3)

- b) Summarise the advantages of stopping smoking in terms of the percentage reduction in risk with passing time.

(3)

- c) Explain how smoking can harm the unborn child of a non-smoking mother to be.

(2)

Question continued...

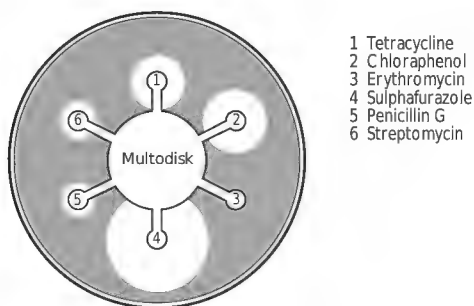
- d)** Give an account of the problems involved in demonstrating the link between smoking and lung cancer.

(7)

Total 15

Question 10

Study the diagram below which illustrates the effect of different anti-bacterial agents on a culture of bacteria grown on an agar plate. The sterile paper 'multidisc' has 6 arms and the tip of each is impregnated with a different anti-bacterial agent. Anti-bacterial agents 1, 3, 5, and 6, are antibiotics.



- a) i)** Explain why it is essential for the purposes of the experiment, that the multodisc is sterile.

(2)

- ii)** Explain why the type of bacterial culture used on the agar plate is significant.

(2)

- b)** Describe and explain the appearance of the agar plate as shown in the diagram.

[illegible]

(7)

Total 11

Assessment Grid

3 - Pathogens and Disease Year Set I

	Question Number										
Specification Section	1	2	3	4	5	6	7	8	9	10	Total
3.1 Microorganisms	6										6
3.2 Parasites		6									6
3.3 Immunology			7				6				13
3.4 Mitosis				5							5
3.5 DNA					7						7
3.6 Gene technology						7					7
3.7 CHD and cancer									14		14
3.8 Diagnosis								6			6
3.8 Drugs										11	11
											Total 75

	Question Number										
Assessment Objective	1	2	3	4	5	6	7	8	9	10	Total
AO1 Knowledge with Understanding	5	2	5	3	6	4	4	2	8	4	43
AO2 Application/Analysis/ Evaluation	1	4	2	2	1	3	2	4	6	7	32
											Total 75

Mark Schemes for Question Paper

3- Pathogens and Disease Year Set I

Instructions

; = 1 mark / = alternative response

Question I

Study the sigmoid growth curve of a bacterial population incubated at 20oC below and then answer the questions that follow.

- a) Mark and label the following phases on the curve of the graph:
decline phase, log phase, lag phase, and stationary phase. (2)
- b) Describe what is occurring in the bacterial population during the log phase.
asexual reproduction by binary fission;
giving a short doubling time;
compound interest / exponential growth (2)
- c) Describe what is occurring in the bacterial culture during the decline phase.
depletion of nutrients;
accumulation of waste products;
death rate greater than reproductive rate; (2)

Total 6

Question 2

A simplified diagram of the malarial parasite (*Plasmodium*) life cycle is shown below.

- a) Explain why the female mosquito injects saliva into the individual on which she is going to feed.
it contains an anti-coagulant to prevent blood clotting; (1)
- b) A parasite must be able to survive the hostile environment within the host. Describe the way in which the blood stream could be considered:
- i) an ideal environment for the malarial parasite;
constant optimum temperature of 37°C / constant supply of nutrients / protected; (1)
- ii) a hostile environment for the malarial parasite;
anti-bodies / immune system will attack them; (1)
- c) Describe those features of the malarial parasite's life cycle that could be considered to be associated with the infection of a new host.
- sexual stages in the mosquito vector which actively seeks hosts;
migration to the saliva glands of mosquito ensures introduction into host;
strong powers of asexual reproduction increases chances of being picked up by vector;
reservoirs of infection in liver cells protected from immune system;
and drugs;
result in waves of emergence into blood stream long after initial infection; (3)

Total 6

Question 3

Read the following passage, and using information from the passage and your own knowledge answer the questions that follow.

- a) i) antigen
compound (typically protein or glycoprotein) which is recognised and attacked by antibodies; (1)
- ii) antibody
compound (protein) which recognises and attacks antigens; (1)
- iii) pathogen
disease causing organism; (1)
- b) Describe the relationship between pathogens and antigens.
pathogens have specific protein / glycoproteins on their cell surface membranes;
which act as antigens to antibodies in the host; (2)
- c) Explain how the immunity to some diseases lasts a life time, but some illnesses can be suffered more than once
memory cells vary in longevity;
long lasting (confer life time immunity) / short lasting allow further invasions; (2)

Total 7

Question 4

Study the illustration below of a stained cells from a squashed root tip of a plant, showing various stages of mitosis, and then answer the questions that follow.

- a) i) Indicate with an X on the illustration a cell which is showing the separation of chromatids (1)
- ii) Indicate with a Y on the illustration a cell which is showing the chromosomes lining up on the equator of the nuclear spindle.
nucleus correctly identified (1)
- b) Explain why the specimen was **squashed** and not sectioned.
squashing separates out the individual chromosomes;
in one plane so all can be seen;
sectioning would only catch parts of chromosomes; (2)
- c) Explain why plant root tips are good source of cells undergoing mitosis.
growing regions / apical meristem just behind tip;
produce nuclei by mitosis in all directions; (1)

Total 5

Question 5

Study the diagram below of replicating DNA and then answer the questions that follow.

- a) On the diagram:
- i) label the two new strands of DNA; (1)
 - ii) mark the position of the next hydrogen bonds to be broken; (1)
 - iii) indicate the direction in which the new strands of DNA will be formed; (1)
- b) With reference to the diagram:
- describe what is meant by semi-conservative replication;
 - each daughter DNA double helix;
 - only one strand copied;
 - has conserved one strand of the original DNA double helix; (3)
- c) Name an enzyme involved in catalysing the chemical reactions of DNA replication
DNA polymerase (1)

Total 7

Question 6

The table below summarises the effects of two antibiotics on genetically modified (engineered) bacteria, which had been exposed to plasmids containing either the two genes for resistance to the two antibiotics in the table, or the gene for resistance to one antibiotic, as well as the gene for human insulin.

- a) i) Describe the nature of plasmids.
circular / ring like DNA in bacterial cytoplasm
with few genes; (1)
- ii) Describe and explain the treatment to which bacteria can be exposed that encourages the uptake of plasmids.
ice-cold calcium chloride;
after 15 minutes in ice 90 seconds at 42°C
agitation; (2)
- iii) From the results shown in the table describe the state of bacteria A, B, and C in terms of which plasmids they have taken up.
A none taken up
B unaltered plasmids taken up
C plasmids with gene for human insulin taken up; (1)
- b) Given the results in the table, briefly describe how bacteria carrying the gene for human insulin could be isolated from a mixed culture of A,B and C.
plate out on agar plates containing ampicillin;
B and C will grow;
replica plate onto agar plates containing both antibiotics;
only B colonies will grow on this;
return to previous plate and take sample from colony(ies) of C; (3)

Total 7

Question 7

- a) i) The majority (70%) of white cells in the blood are phagocytic granulocytes. Describe the process of phagocytosis.
- pseudopodia / cytoplasmic extensions surround particle;
into vacuole / phagosome;
lysosomes aggregate around and coalesce with vacuole;
particle digested by enzymes; (3)
- ii) Granulocytes only survive in the blood and tissue fluids for up to about 7 days. Suggest a reason why being phagocytic might contribute to this relatively short existence span.
- digestive enzymes include proteases which leak into cytoplasm;
autodigestion of phagocyte; (1)
- b) Explain how the granulocytes can concentrate around points of infection outside of the bloodstream.
- squeeze out between cells of endothelium of capillary walls;
into tissue fluid;
chemotaxis to damaged tissue; (2)

Total 6

Question 8

- a) Name two characteristics of enzymes that enable them to be used as analytical reagents.
high sensitivity;
specificity; (2)
- b) The enzyme glucose oxidase catalyses the conversion of glucose to gluconic acid. Glucose oxidase, immobilised in a semi-conducting silicon chip, can give a measure of the level of blood glucose by the generation of a small electric current which can be converted to a liquid crystal display.
- i) Name a condition in which the accurate measurement of blood glucose is vital.
diabetes mellitus; (1)
- ii) Explain how the conversion that glucose oxidase catalyses can result in an accurate measure of blood glucose level.
gluconic acid dissociates to release hydrogen ions;
hydrogen ions result in an electrical current;
proportional to the amount of acid;
in turn proportional to the amount of glucose present / converted; (3)

Total 6

Question 9

Study the data presented below on smoking and lung cancer, and answer the following questions.

- a) Suggest an explanation for why there is such a high death rate from lung cancer in males between 55-64 years of age.
length of time smoking increases risk;
40 to 50 years ago smoking widely accepted;
risks only beginning to be understood then;
females smoked less than males in past;
cancer primarily a disease of old age; (3)
- b) Summarise the advantages of stopping smoking in terms of the percentage reduction in risk with passing time.
47% / 44% reduction in risk after stopping for 10 years;
86% / 79% reduction in risk after stopping for 10 years;
(first figures of pairs allow for 10 non-smoking fatalities per 100 000)
risk never returns to normal; (3)
- c) Explain how smoking can harm the unborn child of a non-smoking mother to be.
passive smoking by mother;
nicotine and carcinogens into bloodstream of mother;
cross placenta and damage foetus; (2)
- d) Give an account of the problems involved in demonstrating the link between smoking and lung cancer.
correlation does not prove cause and effect;
minority of smokers (35%-50%) die of lung cancer;
multi-factorial causes of cancer;
including inherited propensity;
many other atmospheric pollutants implicated;
epidemiological studies required with large numbers for statistical significance;
over long periods of time / longitudinal studies;
difficult to ensure accurate answers from smokers about their habit;
animal experiments do not always translate to humans; (7)

Total 15

Question 10

Study the diagram below which illustrates the effect of different anti-bacterial agents on a culture of bacteria grown on an agar plate. The sterile paper 'multodisc' has 6 arms and the tip of each is impregnated with a different anti-bacterial agent. Anti-bacterial agents 1, 3, 5, and 6, are antibiotics.

- a) i) Explain why it is essential, for the purposes of the experiment, that the multodisc is sterile.
to ensure that no 'foreign' microorganisms are introduced into the experimental culture;
which could compete with experimental culture;
for nutrients and space or produce antibiotics if fungi (2)
- ii) Explain why the type of bacterial culture used on the agar plate is significant.
different bacteria have different susceptibilities to different antibiotics;
e.g. penicillin ineffective against TB;
cannot extrapolate from reaction of one bacterium to reaction of another; (2)
- b) Describe and explain the appearance of the agar plate as shown in the diagram.
the experimental culture is spread evenly over the plate;
except around discs 1, 2, 4, 5, and 6;
around these are clear circular patches;
due to diffusion of the anti-bacterial agents from the discs;
killing the bacteria in these areas
except one (no. 3) to which the bacteria must be resistant;
variation in size of other circles due to variation in bacterial resistance; (7)

Total 11

Biology



1 - Molecules, Cells & Systems

2 - Making Use of Biology

3 - Pathogens & Disease

Complete with Assessment Grids & Mark Schemes

FELTHAM PRESS

Examination Style Question Papers

Name: _____

Unit 1 - Molecules, Cells and Systems

Year Set 2

Time allowed 1 hour 30 minutes

Instructions:

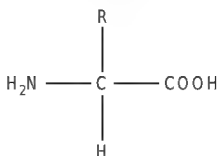
- Answer **all** the questions in the spaces provided.

Information

- Mark allocations are shown in brackets.
- The maximum mark for this paper is 75.
- just over half of the marks (43/75) will be for knowledge and understanding; just under half of the marks (32/75) will be for application of knowledge and understanding, analysis and evaluation;
- Quality of Written Communication will be assessed in answers to these questions.
- Scientific terminology should be used where appropriate.
- Calculators may be used.

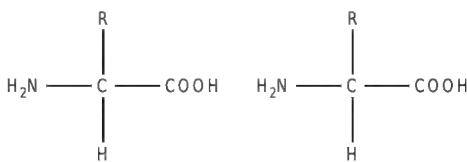
Question 1

The structural formula of an amino acid is shown below.



- a) There are about 20 different types of amino acid to be found in proteins of living organisms, explain how the structural formula shown above applies to them all even though they are different.

(2)



- b) On the diagram below show how two amino acids can combine.

(2)

- c) Name the type of reaction that results in the combination shown in section (b).

(1)

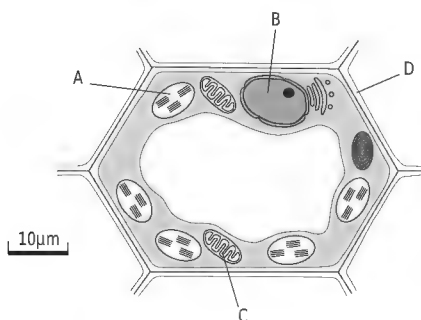
- d) Explain how only about 20 different types of amino acids can form the huge variety of proteins found in living organisms.

(3)

Total 8

Question 2

Study the diagram of a generalised plant cell as seen under the electron microscope and



answer the following questions.

- a) Identify structures A to D

A _____
B _____
C _____
D _____

(4)

- b) If cells such as the one illustrated were treated in the appropriate way and subjected to differential ultracentrifugation, in what order would structures A, B, and C separate out?

(1)

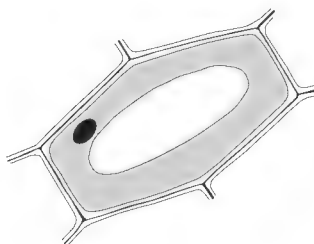
- c) Name three factors on which their separation depends.

(3)

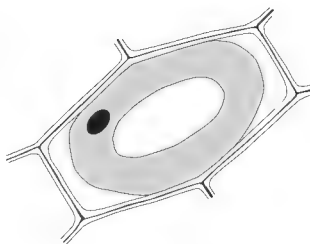
Total 8

Question 3

The diagrams below show two plant cells, each bathed in a different external solution.



Cell A



Cell B

- a) i) Name the cell which is in a solution more dilute than the cell contents.

(1)

- ii) Describe the appearance of the cell which is in a solution more concentrated than the cell contents.

(2)

- b) Describe and explain what would happen to animal cells e.g. human red blood cells if they are placed in a solution with a water potential greater than their contents?

(3)

Total 6

Question 4

a) Describe what is meant by the following terms with regard to the uptake of substances by cells:

i) facilitated diffusion

(2)

ii) active transport

(3)

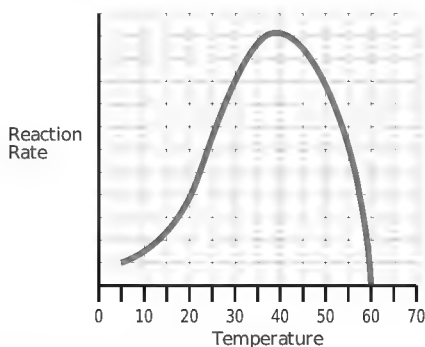
b) Explain why, when respiration is inhibited in cells bathed in pure water, substances, especially ions, are released from the cells.

(4)

Total 9

Question 5

The graph below shows the effect of changing temperature on the rate of an enzyme controlled reaction.



- a) i) Identify the optimum temperature for this enzyme.

(1)

- ii) Explain, from the basis of this optimum temperature, whether the enzyme was obtained from a human, or from a bacterium living in a hot spring with a temperature of 80°C

(2)

- b) i) Explain why the rate of reaction slows at temperatures below the optimum.

(2)

- ii) Explain why the rate of reaction slows at temperatures above the optimum..

(2)

- c) A graph of rate of enzyme reaction against pH would have a similar shape to the one above, but there is an important difference between the state of the enzyme at the lowest temperature and the state of the enzyme at the lowest pH. Explain this difference.

(3)

Total 10

Question 6

- a) i) Describe what is meant by the term 'tissue'.

(2)

- ii) Describe the essential features of the alveolar epithelium as a surface over which gas exchange takes place.

(2)

- b] Explain how the structure of red blood cells is adapted to their function of oxygen transport.

(3)

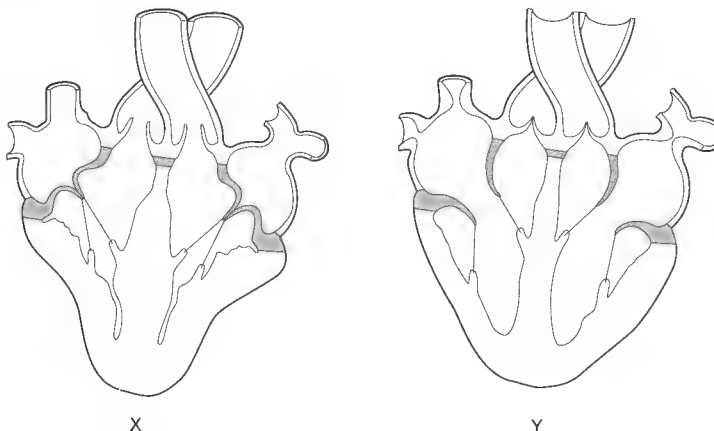
- c) Mature red blood cells, like prokaryotic cells, lack a nucleus. Explain why they are still considered to be eukaryotic cells.

(2)

Total 9

Question 7

Study the diagrams below showing the state of the heart at two stages in the cardiac cycle, and answer the following questions.



- a) i) Identify the diagram in which the ventricles are relaxed.

(1)

- ii) By means of a cross on either diagram identify the chamber in which the pressure is highest.

(1)

- iii) By means of arrows on the two diagrams above indicate the direction of the blood flow into and out of the heart.

(1)

- b) i) With reference to diagram Y explain why the pocket valves are shut during this stage of the cardiac cycle.

(3)

- c) i) Account for the difference between the position of the atrio-ventricular valves in diagrams X and Y

(2)

- ii) Explain how these valves are prevented from opening in the wrong direction.

(1)

Total 9

Question 8

Read the following passage.

Living cells, tissues and small organisms can be observed with the light (optical) microscope. Sometimes their visibility can be improved by the use of stains that do not harm living material (vital stains). More detail is revealed if the material is fixed (killed), sectioned, and stained. Staining allows cell structures to be distinguished from each other as a result of variation in the amount of stain that they take up. These processes, as well as dehydration, which are necessary when making permanent slides, involve treatments that distort the cells in some way, producing the appearance of false structures or artifacts. The maximum effective magnification for the light (optical) microscope is about 1500, beyond this no further detail is revealed.

Using information from the passage and your own knowledge answer the following questions.

- a) Explain why staining improves the visibility of cells, tissues and small organisms under the light (optical) microscope.

(1)

- b)** Give an example of an artefact that you might see under the light (optical) microscope.

(1)

- c)** Describe what is meant by the term resolving power in relation to microscopes.

(1)

- d) Describe how you would prepare a temporary mount of some biological material, using a simple staining technique. Explain the reason for each of your actions.

[illegible]

(5)

Total 8

a) Explain how the cardiac output is increased in this way.

b) The proportion of the cardiac output circulating to the skeletal muscles can increase from around 20% when at rest to around 85% during maximal aerobic exercise, describe why this occurs and how it is achieved.

[illegible]

Total 8

Assessment Grid

Unit 1 - Molecules, Cells and Systems Year Set 2

Specification Section	Question Number									Total
	1	2	3	4	5	6	7	8	9	
11.1 Cell Structure						2		8		10
11.2 Ultrastructure		8								8
11.3 Membrane & Cell Transport			6	9						15
11.4 Biological Molecules	8									8
11.5 Enzymes					10					10
11.6 Tissue and Organs						7				7
11.7 Blood System							9			9
11.8 Heart									8	8
Total										75

Assessment Objective	Question Number									Total
	1	2	3	4	5	6	7	8	9	
AO1										
Knowledge with Understanding	3	8	4	5	7	4	3	4	4	42
AO2										
Application/Analysis/Evaluation	5		2	4	3	5	6	4	4	33
Total										75

Mark Schemes for Question Paper

Unit 1 - Molecules, Cells and Systems Year Set 2

Instructions

; = 1 mark / = alternative response

Question 1

The structural formula of an amino acid is shown below.

- a) There are about 20 different types of amino acid to be found in proteins of living origin, explain how the structural formula shown above applies to them all even though they are different.
the R group varies in structure;
providing the variation between them; (2)
- b) On the diagram below show how two amino acids can combine.
showing removal of elements of water;
formation of peptide bond CO-NH; (2)
- c) Name the type of reaction that results in the combination shown in section (b).
condensation reaction; (1)
- d) Explain how only about 20 different types of amino acids can form the huge variety of proteins of living origin.
any combination of different amino acids;
any number of each type of amino acid;
resulting in a huge variety of different interactions between R groups etc;
leading to variety of primary, secondary and tertiary structures; (3)

Total 8

Question 2

- a) Identify structures A to D

A = Chloroplast

B = Nucleus

C = Mitochondrion

D = Cell wall

(4)

- b) If cells such as the one illustrated were treated in the appropriate way and subjected to differential ultracentrifugation, in what order would structures A, B, and C separate out.
nucleus, chloroplast, mitochondrion;

(1)

- c) Name three factors on which their separation depends.

mass;

density compared to the suspending solution;

the gravitational force to which they are subjected;

(3)

Total 8

Question 3

The diagrams below show two plant cells, each bathed in a different external solution.

- a) i) Name the cell which is in a solution more dilute than the cell contents.
cell A; (1)
- ii) Describe the appearance of the cell which is in a solution more concentrated than the cell contents.
the cell membrane is withdrawn from the cell wall;
the central vacuole is smaller than in cell A; (2)
- b) Describe and explain what would happen to animal cells e.g. human red blood cells if they are placed in a solution with a water potential greater than their contents?
water would enter by osmosis (endosmosis);
cells would increase in volume (swell);
cell membrane would be stretched;
and burst if water potential gradient high enough; (3)

Total 6

Question 4

a) Describe what is meant by the following terms with regard to the uptake of substances by cells:

i) facilitated diffusion

diffusion down a diffusion gradient;

from higher concentration to lower concentration;

speeded up/ made easier by the presence of special carriers in the membrane;

(2)

ii) active transport

transport against the prevailing diffusion gradient;

from lower concentration to higher concentration;

involving carriers in cell membrane;

using energy from respiration / ATP;

(3)

b) Explain why, when respiration is inhibited in cells bathed in pure water, substances, especially ions, are released from the cells.

in pure water the diffusion gradient for ions is from the more concentrated cell contents to the pure water;

this situation is maintained by active ion uptake;

using energy from respiration / ATP;

if respiration is inhibited active uptake is reduced and substances diffuse out of the cell into the surrounding water;

if respiration inhibited completely, membrane breaks down and all cell contents are released into the surrounding water;

(4)

Total 9

Question 5

The graph below shows the effect of changing temperature on the rate of an enzyme controlled reaction.

- a) i) Identify the optimum temperature for this enzyme.
37°C; (1)
- ii) Explain, from the basis of this optimum temperature, whether the enzyme was obtained from a human, or from a bacterium living in a hot spring with a temperature of 80°C.
human body temperature 37°C;
optimum temperature of enzyme 37°C;
if from hot spring bacterium would have higher optimum temp; (2)
- b) i) Explain why the rate of reaction slows at temperatures below the optimum.
rate of collisions of reacting molecules temperature dependent;
the lower the temperature the lower the rate of collisions;
lowers the rate of all chemical reactions including enzyme catalysed; (2)
- ii) Explain why the rate of reaction slows at temperatures above the optimum.
enzyme molecules progressively denatured / disrupted;
cannot form enzyme / substrate complexes;
rate of reaction slows despite increased rate of collision; (2)
- c) A graph of rate of enzyme reaction against pH would have a similar shape as the one above, but there is an important difference between the state of the enzyme at the lowest temperature and the state of the enzyme at the lowest pH. Explain this difference.
at lowest temperature enzyme not denatured;
reversible;
at lowest pH enzyme denatured;
irreversible; (3)

Total 10

Question 6

- a) i) Describe what is meant by the term 'tissue'.
a collection/aggregation/association of similar cells;
specialised for/carrying out a common function; (2)
- ii) Describe the essential features of the alveolar epithelium as a surface over which gas exchange takes place.
thin in cross section'
large surface area to volume ratio;
tightly bound together into a membrane;
mucus secreting cells to reduce water loss by evaporation; (3)
- b) Explain how the structure of red blood cells is adapted to their function of oxygen transport.
large surface area to volume ratio;
as a result of their relatively small size;
and their biconcave shape;
as a result of no nucleus;
which also allows whole cell contents to be filled with haemoglobin;
haemoglobin content also maximised by loss of virtually all cell organelles; (3)
- c) Mature red blood cells, like prokaryotic cells, lack a nucleus. Explain why they are still considered to be eukaryotic cells.
have a nucleus when formed;
have membrane bound organelles when formed;
are part of a tissue in mammals; (2)

Total 11

Question 7

Study the diagrams below showing the state of the heart at two stages in the cardiac cycle, and answer the following questions.

- a) i) Identify the diagram in which the ventricles are relaxed.

Y

(1)

- ii) By means of a cross on the diagram identify the chamber in which the pressure is highest.

Cross correctly identifying the left ventricle in diagram X

(1)

- iii) By means of arrows on the two diagrams indicate the direction of the blood flow into and out of the heart.

Direction of flow of blood correctly shown on both diagrams.

(1)

- b) i) With reference to diagram Y explain why the pocket valves are shut during this stage of the cardiac cycle.

by blood falling back down the blood vessels exiting the right and left ventricles (aorta and pulmonary arteries);

as a result of the lower pressure in the empty ventricles;

blood fills 'pockets';

'pockets' block and shut the blood vessels;

(3)

- c) i) Account for the difference between the position of the atrio-ventricular valves in diagrams X and Y in X ventricles contracting forcing blood up;

pushing valves up and shut;

(2)

- ii) Explain how these valves are prevented from opening in the wrong direction.

prevented from opening the wrong way by tendon cords becoming taut;

aided by muscles of ventricular wall exerting a downward pull on tendon cords;

(1)

Total 9

Question 8

Using information from the passage and your own knowledge answer the following questions.

- a) Explain why staining improves the visibility of cells, tissues and small organisms under the light (optical) microscope.
uptake of different amounts of stain by different parts;
determines amount of light transmitted and therefore the appearance; (1)
- b) Give an example of an artefact that you might see under the light (optical) microscope.
air bubble;
crystal of stain;
particle of detritus (1)
- c) Describe what is meant by the term resolving power in relation to microscopes.
the ability to reveal detail;
to be able to distinguish the boundary between adjacent structures (1)
- d) Describe how you would prepare a temporary mount of some biological material, using a simple staining technique. Explain the reason for each of your actions.
specimen that transmits light - transparent, small, thin section, etc;
immerse in stain - preferably in staining dish / not on final slide for cleanliness;
for recommended time interval - for correct density of stain;
wash off excess stain with solvent of stain - to avoid distortions of tissues;
place on a clean dry slide - cleanliness;
check density of staining - not worth mounting if stained incorrectly;
add a drop of mounting fluid (water or glycerol) - improves visibility;
gently lower cover slip - improves visibility and protects lenses of microscope;
(allow reverse technique where specimen is placed on the cover slip and the slide is lowered onto the cover slip)
taking care to avoid forming air bubbles - appear as artifacts and obscure structures;
clean off excess mounting fluid - cleanliness and protect microscope lenses; (5)

Total 8

Question 9

The cardiac output can increase from 5 dm³ (litres) per minute at rest to 30 dm³ (litres) per minute at maximal exercise.

- a) Explain how the cardiac output is increased in this way.

increase in heart rate;

and increase in stroke volume;

(2)

- b) The proportion of the cardiac output circulating to the skeletal muscles can increase from around 20% when at rest to around 85% during maximal aerobic exercise, describe why this occurs and how it is achieved.

active muscles demand more oxygen than when at rest;

for the release of energy;

by aerobic respiration;

also active muscles demand more nutrients (glucose);

produce more waste (heat, carbon dioxide, and lactate) to be removed;

blood is redirected / shunted;

by means of opening and shutting capillary beds;

by control of the precapillary sphincter muscles;

capillary beds opened in active muscles;

capillary beds closed in tissues and organs not directly involved in exercise;

(6)

Total 8

Examination Style Question Papers

Name: _____

Unit 2 - Applied Biology **Year Set 2**

Time allowed | hour 30 minutes

Instructions:

- Answer **all** the questions in the spaces provided.

Information

- Mark allocations are shown in brackets.
- The maximum mark for this paper is 75.
- just over half of the marks (43/75) will be for knowledge and understanding; just under half of the marks (32/75) will be for application of knowledge and understanding, analysis and evaluation;
- This type of paper would carry 35 per cent of the total marks for AS level, and 17.5 per cent of the total marks for A level.
- Quality of Written Communication will be assessed in answers to these questions.
- Scientific terminology should be used where appropriate.
- You may use a calculator.

Question 1

a) Immobilised enzymes are of great industrial importance.

i) Describe three ways in which enzymes can be 'immobilised' for use in industrial processes.

(3)

ii) Explain why the immobilisation of proteases is particularly important in such processes.

(1)

b) Explain why enzymes used in industrial processes require a high degree of thermostability.

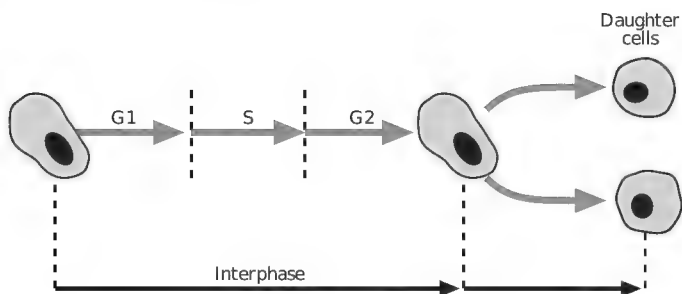
(3)

Total 7

Question 2

Study the diagram of the cell cycle of a body cell of a multicellular organism and answer the questions that follow.

- a) On the diagram label the following:



i) the period during which mitosis occurs; (1)

ii) the period in which the amount of DNA doubles (replicates); (1)

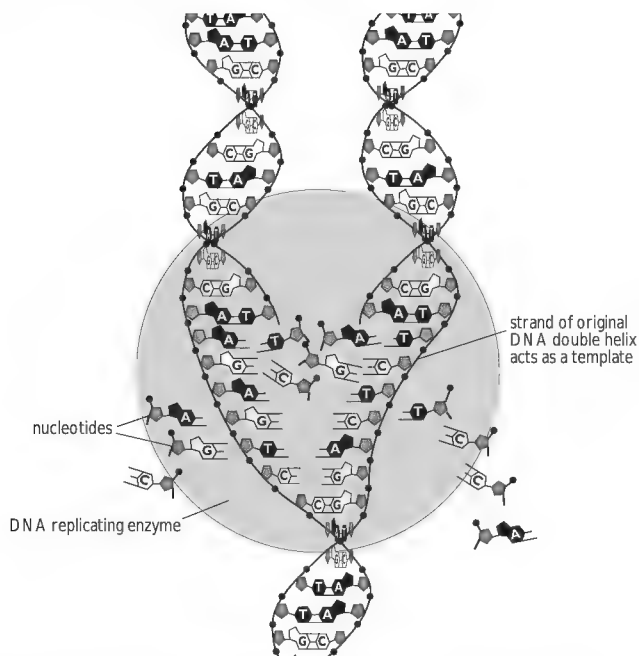
iii) the point past which the cell will complete the cycle and enter division; (1)

- b) Explain why it is necessary for the amount of DNA to double during this cell cycle;

(3)

Total 6

Question 3



Study the diagram above of replicating DNA and answer the following questions.

a) On the diagram:

i) label the two new strands of DNA;

ii) label with an X a pair of complementary bases;

iii) indicate with an arrow the direction of formation of the new strands of DNA;

(3)

b) With reference to the diagram:

i) describe what is meant by semi-conservative replication;

(2)

ii) list in the correct order the bases of the next three nucleotides that will pair up with those of the lower strand;

(1)

c) Name an enzyme involved in catalysing the chemical reactions of DNA replication

(1)

Total 7

Question 4

The production of human insulin (and other proteins) from single cell organisms such as bacteria. requires as a first step the isolation of the relevant human gene.

- a) Describe which cells of the human body would be best to use as a source of the human gene for insulin, and explain why.

(2)

- b) i) Explain why it is better to isolate the mRNA from these cells, and not to try and isolate the actual length of DNA that codes for the insulin.

(2)

- ii) Describe the action of the enzyme reverse transcriptase in this process of gene isolation.

(1)

Total 5

Question 5

Read the following passage, and using information from the passage and your own knowledge answer the following questions.

B-lymphocytes form memory cells after meeting an antigen, e.g. a pathogen, in the body. These memory cells allow the body to 'remember' antigens it has met before and produce a much faster immune response to them the second time around.

When they do meet a familiar antigen, B-lymphocyte memory cells divide and form new antibody producing plasma cells. Some memory cells always remain so that the body can respond in this way to any number of future infections with the same antigen. The person is said to be resistant or immune to that disease. The immunity to some diseases lasts a life time, but some illnesses can be suffered more than once.

Larger amounts of antibodies are produced more rapidly by B-lymphocytes in the secondary response than in the first (or primary) response to a pathogen.

a) Describe what is meant by terms:

i) antigen

(1)

ii) antibody

(1)

iii) pathogen

(1)

b) Describe the relationship between pathogens and antigens.

(2)

c) Explain how the immunity to some diseases lasts a life time, but some illnesses can be suffered more than once

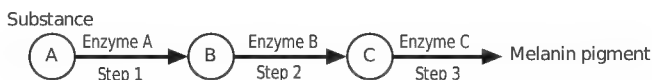
(3)

Total 8

Question 6

Enzymes are proteins whose synthesis is controlled by DNA. Enzymes control metabolic pathways within the cells of the body and thus influence the phenotype of an organism. A simple diagram of a metabolic pathway is shown below

a)



Distinguish between:

i) intracellular enzymes and extracellular enzymes

(2)

ii) genotype and phenotype;

(3)

b) Describe the phenotype which will result if the enzyme controlling Step 1 does not function.

(1)

c) A length of DNA which codes for a protein is known as a gene. Genes can be dominant ie only one copy per diploid set of chromosomes in each cell is required to exert its effect, or recessive where the effect is only exerted if two copies are present. Explain these observations in terms of protein enzymes controlling metabolic pathways.

(4)

Total 10

Question 7

- a) Describe one similarity between organic and inorganic fertilisers.

(1)

- b) In the table provided, list four advantages and two disadvantages of organic fertilisers.

Organic fertilisers	
Advantages	
1	
2	
3	
4	
Disadvantages	
1	
2	

(6)

- c) Explain why inorganic fertilisers represent a pollution load on the environment, whereas organic fertilisers do not

(3)

Total 10

Question 8

Sorghum (the source of millet seed) grows in hot, dry conditions, and has typical xerophytic modifications, including an extensive root system, a thick cuticle, a reduced number of sunken stomata, and the ability to tolerate high temperatures.

- a) Describe the role of xerophytic modifications.

(3)

- b) Describe a special feature of the Sorghum cells which enable the plant to tolerate high temperatures

(1)

- c) Explain how each special feature of the leaves and the roots of Sorghum adapt it to hot dry conditions.

(7)

Total 11

Question 9

The manipulation and control of reproduction in domestic animals is of great commercial interest. One example is seen in the use of Bovine somatotrophin (BST) otherwise known as bovine growth hormone. When BST is injected into dairy cows their milk yield increases by up to 20%

- a) i)** Describe how the hormone could affect the animal's metabolism to produce the increase in milk yield.

(3)

- ii)** Given a constant demand for milk, suggest the effect that the use of BST will have on dairy herds.

(1)

- iii) Describe an important aspect of milk as a product, in addition to that of yield.

(1)

- b)** Give a short account of the criticisms that could be made against the use of BST in dairy cows.

[illegible]

(6)

Total 11

Assessment Grid

Unit 2 - Applied Biology Year Set 2

Specification Section	Question Number									Total
	1	2	3	4	5	6	7	8	9	
11.1 Enzymes	7					2				9
11.2 Mitosis		6								6
11.3 DNA			7			8				15
11.4 Gene technology				5						5
11.5 Forensic science					8					8
11.6 Plant growth							10	11		21
11.7 Biotechnology/Reproduction									11	11
Total										75

Assessment Objective	Question Number									Total
	1	2	3	4	5	6	7	8	9	
AO1										
Knowledge with Understanding	4	3	5	3	5	6	7	5	5	43
AO2										
Application/Analysis/Evaluation	3	3	2	2	3	4	3	6	6	32
Total										75

Mark Schemes for Question Paper

Unit 2 - Applied Biology Year Set 2

Instructions

; = 1 mark / = alternative response

Question 1

- a) Immobilised enzymes are of great industrial importance.
- i) Describe three ways in which enzymes can be 'immobilised' for use in industrial processes.
- adsorption onto resins;
covalent attachment to collagen or synthetic polymers;
lattice entrapment in silica gel;
microencapsulation in alginate or polyacrylamide;
precipitation by direct cross linking; (3)
- ii) Explain why the immobilisation of proteases is particularly important in such processes.
- enzymes are proteins and proteases would digest themselves in solution; (1)
- b) Explain why enzymes used in industrial processes require a high degree of thermostability.
- many industrial process involving enzymes require high temperature;
to ensure rapid and efficient processes;
enzymes denatured at particular temperatures;
expensive to keep renewing enzymes if denatured;
process flow also interrupted; (3)

Total 7

Question 2

Study the diagram of the cell cycle of a body cell of a multicellular organism and answer the following questions.

- a) On the diagram label the following:
- i) the period in which mitosis occurs;
period identified correctly; (1)
 - ii) the period in which the amount of DNA doubles (replicates);
S phase; (1)
 - iii) the point past which the cell will complete the cycle and enter division;
Start of S phase; (1)
- b) Explain why it is necessary for the amount of DNA to double during this cell cycle;
two new daughter body cells are produced;
daughter body cells need to be genetically identical;
doubling (replication) of the DNA results in genetically identical daughter cells (nuclei)
maintains constancy of chromosome number; (3)

Total 6

Question 3

Study the diagram below of replicating DNA and answer the following questions.

- a) On the diagram:
- i) label the two new strands of DNA;
 - ii) label with an X on the diagram a pair of complementary bases;
 - iii) indicate with an arrow the direction of formation of the new strands of DNA; (3)
- b) With reference to the diagram:
- i) describe what is meant by semi-conservative replication;
each daughter DNA double helix;
has conserved one strand of the original DNA double helix; (2)
 - ii) list the bases of the next three nucleotides that will pair up with those of the lower strand;
C, G, T (allow in reverse order) (1)
- c) Name an enzyme involved in catalysing the chemical reactions of DNA replication
DNA polymerase (1)

Total 7

Question 4

The production of human insulin (and other proteins) from single cell organisms such as bacteria, requires as a first step the isolation of the relevant human gene.

- a) Describe which cells of the human body it would be best to use as a source of the human gene for insulin, and explain why.
beta cells of Islets of Langerhans;
specialised for the production of insulin and therefore rich in the appropriate mRNA; (2)
- b) i) Explain why it is better to isolate the mRNA from these cells, and not to try and isolate the actual length of DNA that codes for the insulin.
DNA of these cells contains all genes of organism;
much mRNA in these cells is for the production of insulin; (2)
- ii) Describe the action of the enzyme reverse transcriptase in this process of gene isolation.
copies DNA from mRNA; (1)

Total 5

Question 5

Read the following passage, and using information from the passage and your own knowledge answer the following questions.

- a) Describe what is meant by terms:
- i) antigen
compound (typically protein or glycoprotein) which is recognised and attacked by antibodies; (1)
 - ii) antibody
compound (protein) which recognises and attacks antigens; (1)
 - iii) pathogen
disease causing microorganism; (1)
- b) Describe the relationship between pathogens and antigens.
pathogens have specific protein / glycoproteins on their cell surface membranes;
which act as antigens to antibodies in the host; (2)
- c) Explain how the immunity to some diseases lasts a life time, but some illnesses can be suffered more than once
memory cells vary in longevity;
long lasting (confer life time immunity);
short lasting allow further invasions; (3)

Total 8

Question 6

Enzymes are proteins whose synthesis is controlled by DNA. Enzymes control metabolic pathways within the cells of the body and thus influence the phenotype of an organism. A simple diagram of a metabolic pathway is shown below

- a) Distinguish between:
- i) intracellular enzymes and extracellular enzymes;
intracellular enzymes remain inside cells (control metabolic pathways);
extracellular enzymes secreted from cells (e.g. digestive enzymes); (2)
 - ii) genotype and phenotype;
genotype - the genetic content of the nuclei of the cell(s) of an organism;
phenotype - the expressed reality of the genotype;
phenotype = genotype + environment; (3)
- b) Describe the phenotype which will result if the enzyme controlling Step 1 does not function.
no melanin pigment;
albino;
- c) A length of DNA which codes for a protein is known as a gene. Genes can be dominant ie only one copy per diploid set of chromosomes in each cell is required to exert its effect, or recessive where the effect is only exerted if two copies are present. Explain these observations in terms of protein enzymes controlling metabolic pathways.
genes code for proteins which catalyse specific reactions in metabolic pathways;
one copy of the active gene is sufficient to produce the enzyme;
therefore metabolic pathway functions;
enzyme must be absent before pathway is interrupted
two non-functional versions of the gene result in no enzyme (recessive); (4)

Total 10

Question 7

- a) Describe one similarity between organic and inorganic fertilisers.
both supply plant nutrients e.g. inorganic ions; (1)
- b) In the table provided, list four advantages and two disadvantages of organic fertilisers.
- Organic fertilisers**
- Advantages**
- cheap;
produced in situ;
improve soil structure;
slow release of nutrients;
less danger of leaching;
darken soil increases heat absorption;
- Disadvantages**
- unknown composition;
contains pests / weeds / pathogens (6)
- c) Explain why, in comparison to organic fertilisers, inorganic fertilisers represent a pollution load on the environment.
- organic fertilisers recycle nutrients;
inorganic fertilisers produced from mineral deposits;
energy dependent manufacturing processes;
which are also polluting with green house gases;
more easily leached to cause eutrophication; (3)

Total 10

Question 8

Sorghum (the source of millet seed) grows in hot, dry conditions, and has typical xerophytic modifications, including extensive root system, a thick cuticle, a reduced number of sunken stomata, and the ability to tolerate high temperatures.

- a) Describe the role of xerophytic modifications.
to take up and conserve water / efficient water economy of the plant
by reducing transpiration;
mainly from the leaves; (3)
- b) Describe a special feature of the Sorghum cells which enable the plant to tolerate high temperatures
enzymes denatured at higher temperatures;
less easily inhibited /; (1)
- c) Explain how each special feature of the leaves and the roots of Sorghum adapt it to hot dry conditions.
the extensive root system takes up water from a large volume of soil;
wind blow of dry soil only exposes fraction of total root system;
thick cuticle of leaves reduces evaporation of water through it;
thick cuticle also waxy which further reduces evaporation of water through it;
virtually no cuticular transpiration;
reduced number of stomata reduces water loss by evaporation;
sunken stomata reduce water loss by evaporation;
by protecting from air currents;
and allowing build up of diffusion shells of water vapour over stomata; (7)

Total 11

Question 9

The manipulation and control of reproduction in domestic animals is of great commercial interest. One example is seen in Bovine somatotrophin (BST) otherwise known as bovine growth hormone. When injected into dairy cows the milk yield increases by up to 20%

- a) i) Describe how the hormone could affect the animal's metabolism to produce the increase in milk yield.
- increase in appetite;
 - increase in digestion and absorption of end products;
 - increase in availability of nutrients (and water) needed for milk production;
 - mammary glands stimulated to utilise these extra supplies;
- (3)
- ii) Given a constant demand for milk, suggest the effect that the use of BST will have on dairy herds.
- reduced by 20%;
- (1)
- iii) Describe an important aspect of milk in addition to that of yield that must be considered.
- nutrient content;
- (1)
- b) Give a short account of the criticisms that could be made against the use of BST in dairy cows.
- too much milk already being produced;
 - disease of udders (mastitis);
 - use / abuse of more antibiotics;
 - wider effect on antibiotic resistance in human medicine;
 - lifespan of dairy cows reduced;
 - greater stress on animals / only considered as milk machines;
 - metabolism of injected animals can counter compensate;
 - nutrient content of milk altered;
 - if herd sizes not reduced greater pollution from excreta;
 - and methane production;
 - and greater demand for feed;
 - unknown effects may have long term consequences;
 - passage of BST into human food chain;
- (6)

Total 11

Examination Style Question Papers

Name: _____

Unit 3- Pathogens and Disease

Year Set 2

Time allowed | hour 30 minutes

Instructions:

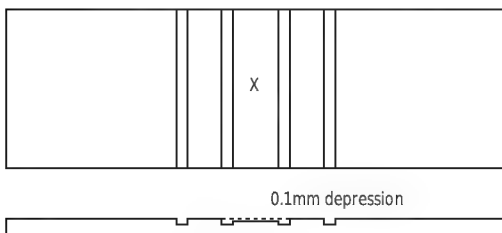
- Answer **all** the questions in the spaces provided.

Information

- Mark allocations are shown in brackets.
- The maximum mark for this paper is 75.
- just over half of the marks (43/75) will be for knowledge and understanding; just under half of the marks (32/75) will be for application of knowledge and understanding, analysis and evaluation;
- This type of paper would carry 35 per cent of the total marks for AS level, and 17.5 per cent of the total marks for A level.
- Quality of Written Communication will be assessed in answers to these questions.
- Scientific terminology should be used where appropriate.
- You may use a calculator.

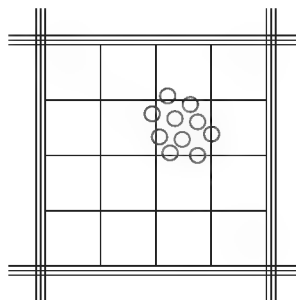
Question 1

When studying populations of microorganisms, such as yeast cells, samples are taken, and the number of yeast cells in a given volume are counted using a counting chamber or haemocytometer.



The counting chamber (haemocytometer). Counting area marked X. A cover slip placed across this area makes a chamber 0.1mm deep.

X



Y

- a) i) On diagram Y, shade / 'colour' in those cells which would be counted as belonging to this square.

(1)

- ii) Explain why counting has to be done in the way that you have indicated in section i).

(1)

- b) i) Each square has sides of 0.05mm, calculate the volume of fluid within one square showing your workings.

(2)

- ii) It is usual to count the cells in 80 such squares, calculate the volume of fluid within 80 squares.

(1)

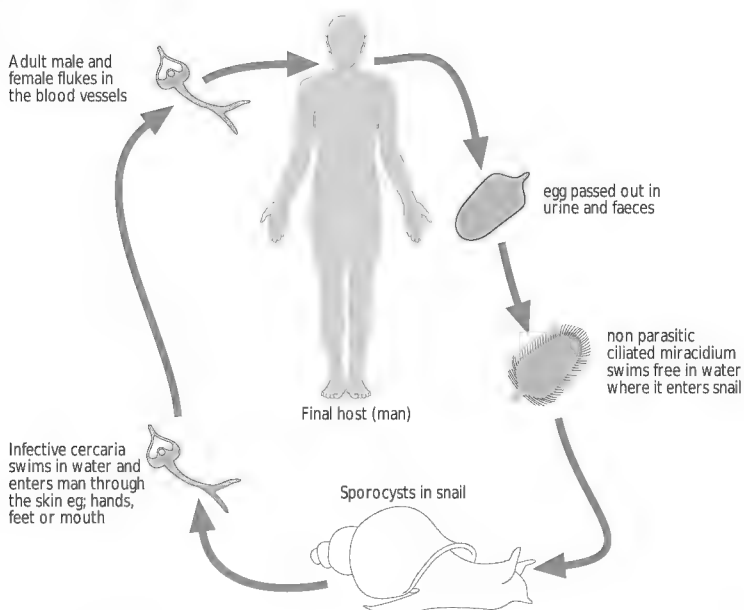
- c) Calculate the number of cells in 1 mm³ of the fluid.

(1)

Total 6

Question 2

Schistosoma is a parasitic flatworm or 'flake' which at one stage in its life cycle lives in the blood stream of humans resulting in the disease Schistosomiasis. A simplified diagram of its life cycle is shown below.



- a) One characteristic of parasites is that they do harm to their host. Suggest two ways in which *Schistosoma* damages its human host.

i) _____

(1)

ii) _____

(1)

- b) *Schistosoma* has separate sexes, whilst close relatives e.g. liver fluke, are hermaphrodite (both male and female organs in each individual). Suggest an advantage for a parasite being a hermaphrodite.

(2)

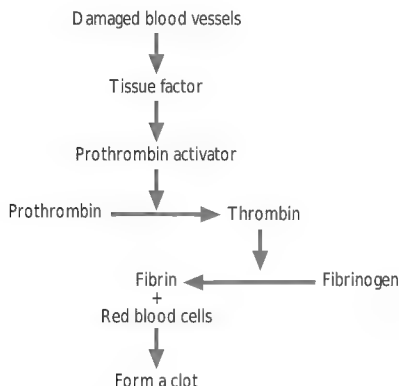
- c) Describe at which point in its life cycle the parasite is most vulnerable, and suggest one method of control that could be applied at this point.

(2)

Total 6

Question 3

Blood clotting involves a chain or 'cascade' of reactions with at least 12 clotting factors involved. A simplified diagrammatic summary of the sequence of reactions leading to the formation of a blood clot is shown below:



- a) i) Identify a soluble protein in the sequence.

(1)

- ii) Identify an insoluble protein in the sequence.

(1)

- b) Describe and explain the effect of the absence of any one of the factors in the chain.

(2)

- c) Name one co-factor (not shown) which is supplied directly from the diet.

(1)

Total 5

Question 4

a) Give brief definitions of the following types of immunity.

i) Natural passive immunity in the unborn child.

(2)

ii) Artificial passive immunity

(1)

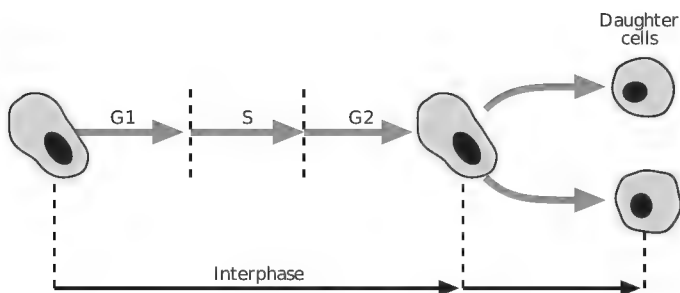
b) With regard to the acquisition of immunity, explain why breast feeding is to be preferred to bottle feeding.

(2)

Total 5

Question 5

Study the diagram of the cell cycle of a body cell of a multicellular organism then answer the questions that follow.



a) On the diagram label the following:

- i) the period in which mitosis occurs; (1)
- ii) the period in which the amount of DNA doubles (replicates); (1)
- iii) the point past which the cell will complete the cycle and enter division; (1)

b) Explain why it is necessary for the amount of DNA to be doubled during this cell cycle;

(3)

Total 6

Question 6

a) Briefly describe the use of the following enzymes in genetic engineering.

i) reverse transcriptase

(2)

ii) restriction endonuclease

(2)

iii) ligase

(2)

b) In the context of gene technology explain why viruses are well suited for their role as 'vectors'.

(2)

Total 8

Question 7

Pancreatitis is an inflammation of the pancreas, the symptoms of which may be mistaken for a perforated peptic ulcer. However, it differs from a peptic ulcer in that the level of amylase in the gut is decreased. and the level of amylase in the blood plasma is raised.

- a)** Explain this difference in symptoms between the two conditions.

(3)

- b)** Patients suffering from pancreatitis are fed intra-venously, with no food or drink by mouth. Explain the reason behind this treatment in terms of normal pancreatic function.

(2)

Total 5

Question 8

Beta blockers are drugs used to reduce chronic high blood pressure (essential hypertension).

- a) Describe exactly what it is that they 'block'.

(1)

- b) One of the major effects of beta blockers is to slow the heart rate. Explain how this would help lower blood pressure.

(2)

- c) Some beta blockers have an additional arteriolar vasodilation action. Explain how this would help lower blood pressure.

(1)

- d) Despite this apparently clear connection between their effects on the heart and blood vessels and the reduction of blood pressure, the exact mode of action of beta blockers in controlling blood pressure is not understood. Suggest why this could be so.

(2)

Total 6

Question 9

- a) The genetic code consists of a sequence of bases on a strand of DNA, and can be represented by a series of letters on a line as shown below

T A C C G T T C T A C C

- i) Describe what the letters represent, and give their names.

(2)

- ii) Draw a similar line with letters to represent the complementary strand of mRNA to the original DNA strand shown at the start of the question.

(1)

- b) The table below shows the genetic code on a strand of mRNA for selected amino acids.

Amino acid	mRNA codons
alanine	GCA, GCC, GCG, GCU
tryptophan	UGG
arginine	AGA, AGG, CGA, CGC, CGG, CGU
lysine	AAA, AAG
methionine	AUG (also acts as the 'start' codon so that every polypeptide chain initially starts with methionine)

Using the information in the table give answers to the following:

- i) explain what is meant by describing the genetic code as 'degenerate';

(2)

- ii) write out the sequence of amino acids coded for by the original strand of DNA shown in part (a) at the start of the question

(2)

Question continued...

(7)

Total 14

Question 10

Study the chart below setting out various heart disease risk factors and answer the following questions.

Age	Sex	Weight	% Animal Fat in Diet	
10–20 = 1	Female under 40 = 1	More than 2kg below ideal weight = 0	No animal fat = 1	
21–30 = 2	Female 40–50 = 2	Within 2kg of ideal weight = 1	10% animal fat = 2	
31–40 = 3	Female over 50 = 3	15kg overweight = 2	20% animal fat = 3	
41–50 = 4	Male = 5	20kg overweight = 3	30% animal fat = 4	
51–60 = 5	Stocky male = 6	25kg overweight = 5	40% animal fat = 5	
61 & over = 8	Bald stocky male = 7	30kg overweight = 7	50% animal fat = 7	Total
Sub-totals				

Exercise	Tobacco Smoking	History of Heart Disease	Blood Pressure	
Hard manual job & exercise = 2	Non smoker = 0	None = 1	Upper reading 100 = 1	
Manual job & moderate exercise = 2	Cigar or pipe smoker = 1	1 Relative over 60 = 2	Upper reading 120 = 2	
Office job & hard exercise = 3	10 cigarettes a day or less = 3	2 Relatives over 60 = 3	Upper reading 140 = 3	
Office job & moderate exercise = 5	20 cigarettes a day = 5	1 Relative under 60 = 4	Upper reading 160 = 4	
Office job & light exercise = 6	30 cigarettes a day = 7	2 Relatives under 60 = 6	Upper reading 180 = 6	
No exercise at all = 8	40 cigarettes a day = 11	3 Relatives under 60 = 7	Upper reading 200 or more = 8	Total
Sub-totals				
				Grand Total

- a) i) Explain why the lowest total score for all the columns on the chart is not zero.

(2)

Question continued...

- ii) Calculate the percentage by which the maximum risk score can be reduced by exercise.**

(2)

- iii)** In the light of your answer to a) ii) above, explain whether you would advise an exercise regime for sedentary people who have been cleared by a physician to carry it out.

(1)

- b) Describe the types of people who would have the highest and lowest risk factors totals, and explain the advice that you would give to the one with the highest risk.

[illegible]

(7)

Total 14

Assessment Grid

Unit 3 - Pathogens and Disease Year Set 2

	Question Number										
Specification Section	1	2	3	4	5	6	7	8	9	10	Total
11.1 Microorganisms	6										6
11.2 Parasites		6									6
11.3 Immunology			5	5							10
11.4 Mitosis					6						6
11.5 DNA									14		14
11.6 Gene technology						8					8
11.7 CHD and cancer										14	14
11.8 Diagnosis							5				5
11.9 Drugs								6			6
											Total 75

	Question Number										
Assessment Objective	1	2	3	4	5	6	7	8	9	10	Total
AO1 Knowledge with Understanding	3	4	3	3	3	6	3	4	7	7	43
AO2 Application/Analysis/ Evaluation	3	2	2	2	3	2	2	2	7	7	32
											Total 75

Mark Schemes for Question Paper

Unit 3- Pathogens and Disease Year Set 2

Instructions

; = 1 mark / = alternative response

Question 1

When studying populations of microorganisms, such as yeast cells, samples are taken, and the number of yeast cells in a given volume are counted using a counting chamber or haemocytometer.

- a) i) On diagram Y, shade / 'colour' in those cells which would be counted as belonging to this square.
those not touching any of the side lines and those touching the top or left-hand sides; (1)
- ii) Explain why counting has to be done in the way that you have indicated in section i).
to ensure that cells are not counted twice; (1)
- b) i) Each square has sides of 0.05 mm, calculate the volume of fluid within one square showing your workings.
 $0.05 \text{ mm} \times 0.05 \text{ mm} \times 0.1 \text{ mm};$
 $= 0.00025 \text{ mm}^3$ (2)
- ii) It is usual to count the cells in 80 such squares. Calculate the volume of fluid within 80 squares.
 $0.00025 \text{ mm}^3 \times 80 = 0.02 \text{ mm}^3$ (1)
- c) Calculate the number of cells in 1 mm^3 of the fluid.
 $10 \times 80 = 800 \text{ in } 0.02 \text{ mm}^3$
 $800 \times 50 = 40\,000$ (1)

Total 6

Question 2

Schistosoma is a parasitic flatworm or 'flake' which at one stage in its life cycle lives in the blood stream of humans resulting in the disease Schistosomiasis. A simplified diagram of its life cycle is shown below.

- a) One characteristic of parasites is that they do harm to their host. Suggest two ways in which Schistosoma damages its human host.
- i) depletes nutrients / oxygen in plasma; (1)
- ii) block capillaries / release toxic waste products; (1)
- b) Schistosoma has separate sexes, whilst very close relatives e.g. liver fluke, are hermaphrodite (both male and female organs in each individual). Suggest an advantage for a parasite being a hermaphrodite.
- effectively doubles the breeding population;
increases successful sexual reproduction; (2)
- c) Describe at which point in its life cycle the parasite is most vulnerable, and suggest one method of control that could be applied at this point.
- when outside the hosts in water;
prevent contamination of water with human excreta;
spray with helminthicides (poison larvae) (2)

Total 6

Question 3

Blood clotting involves a chain or 'cascade' of reactions with at least 12 clotting factors involved. A simplified diagrammatic summary of the sequence of reactions leading to the formation of a blood clot is shown below:

- a) i) Identify a soluble protein in the sequence.
any other than fibrin; (1)
- ii) Identify an insoluble protein in the sequence;
fibrin; (1)
- b) Describe and explain the effect of the absence of any one of the factors in the chain.
absence of a factor in the chain blocks chain;
fibrin not produced;
blood unable to clot / bleeding uncontrolled; (2)
- c) Name one co-factor (not shown) which is supplied directly from the diet.
vitamin K (1)

Total 5

Question 4

- a) Describe briefly how the following types of immunity are achieved.
- i) Natural passive immunity in the unborn child.
the acquisition of ready made antibodies in a natural way;
across the placenta from the mother to the fetus; (2)
- ii) Artificial passive immunity
the acquisition of ready made antibodies in an artificial way;
when infection is too dangerous / acute to wait for development of natural immunity;
in an inoculation as for rabies (1)
- b) With regard to the acquisition of immunity, explain why breast feeding is to be preferred to bottle feeding.
suckling infant gains maternal antibodies in colostrum ('first' milk) of mother;
not present in bottle feeding; (2)

Total 5

Question 5

Study the diagram of the cell cycle of a body cell of a multicellular organism then answer the questions that follow.

- a) On the diagram label the following:
- i) the period in which mitosis occurs;
period identified correctly (1)
 - ii) the period in which the amount of DNA doubles (replicates);
S phase (1)
 - iii) the point past which the cell will complete the cycle and enter division;
start of S phase (1)
- b) Explain why it is necessary for the amount of DNA to be doubled during this cell cycle;
two new daughter body cells are produced;
daughter body cells need to be genetically identical;
doubling (replication) of the DNA results in genetically identical daughter cells (nuclei); (3)

Total 6

Question 6

- a) Briefly describe the use of the following enzymes in genetic engineering.
- i) reverse transcriptase
formation of DNA ('gene');
from mRNA extracted from target cell; (2)
 - ii) restriction endonuclease
hydrolysis at points along nucleic acid;
'cutting' it into specific shorter lengths for isolation of a 'gene'; (2)
 - iii) ligase
rejoins / sticks 'cut' ends of nucleic acids;
used in splicing foreign gene into bacterial plasmid DNA; (2)
- b) In the context of gene technology explain are well suited for their role as 'vectors'.
viruses naturally inject their nucleic acid into the host cell;
carry the engineered gene;
into the host cell; (2)

Total 8

Question 7

Pancreatitis is an inflammation of the pancreas, the symptoms of which may be mistaken for a perforated peptic ulcer. However, it differs from a peptic ulcer in that the level of amylase in the gut is decreased, and the level of amylase in the blood plasma is raised.

- a) Explain this difference in symptoms between the two conditions.
pancreas normally secretes amylase into the gut in pancreatic juice;
in pancreatitis amylase 'escapes' into the plasma;
therefore amylase level drops in gut and increases in plasma;
peptic ulcer an inflammation of the gut wall not related to secretion of amylase; (3)
- b) Patients suffering from pancreatitis are fed intra-venously, with no food or drink by mouth. Explain the reason behind this treatment in terms of normal pancreatic function.
presence of food and certain drinks in gut act as stimuli;
for secretion of pancreatic juice;
not to be encouraged in pancreatitis; (2)

Total 5

Question 8

Beta blockers are drugs used to reduce chronic high blood pressure (essential hypertension).

- a) Describe exactly what it is that they 'block'.
Beta-adrenoreceptors;
in heart and blood vessel walls; (1)
- b) One of the major effects of beta blockers is to slow the heart rate. Explain how this would help lower blood pressure.
slower heart rate in this case = reduced cardiac output;
blood pressure a function of cardiac output and peripheral resistance; (2)
- c) Some beta blockers have an additional arteriolar vasodilation action. Explain how this would help lower blood pressure.
arteriolar vasodilation reduces peripheral resistance;
blood pressure a function of cardiac output and peripheral resistance; (1)
- d) Despite this apparently clear connection between their effects on the heart and blood vessels and the reduction of blood pressure, the exact mode of action of beta blockers in controlling blood pressure is not understood. Suggest why this could be so.
some other effect as a result of combining with beta-adrenoreceptors;
elsewhere in the body - central nervous system; (2)

Total 6

Question 9

- a) The genetic code consists of a sequence of bases on a strand of DNA, and can be represented by a series of letters on a line as shown below

T A C C G T T C T A C C

- i) Describe what the letters represent, and give their names.

nitrogenous bases / nucleotides;

thymine, adenine, cytosine, guanine

(2)

- ii) Draw a similar line with letters to represent the complementary strand of mRNA to the original DNA strand shown at the start of the question.

A U G G C A A G A U G G

(1)

- b) The table below shows the genetic code on a strand of mRNA for selected amino acids.

Amino acid	mRNA codons
alanine	GCA, GCC, GCG, GCU
tryptophan	UGG
arginine	AGA, AGG, CGA, CGC, CGG, CGU
lysine	AAA, AAG
methionine	AUG (also acts as the 'start' codon so that every polypeptide chain initially starts with methionine)

Using the information in the table give answers to the following:

- i) explain what is meant by describing the genetic code as 'degenerate';

some / most amino acids coded for by more than one triplet codon;

e.g. tryptophan and methionine only have one, but alanine has 4, arginine has 6, lysine has 2

(2)

- ii) write out the sequence of amino acids coded for by the original strand of DNA shown above in part (a).

methionine-alanine-arginine-tryptophan

(2)

Question continued...

- c) Phenylketonuria is a serious condition with many effects including mental retardation. In this condition an enzyme involved in the metabolism of the essential amino acid phenylalanine is missing. Given that excess of phenylalanine results in the devastating effects of phenylketonuria, explain how proteins whose synthesis is controlled by DNA, control metabolic pathways and thus influence the phenotype of an organism.

all enzymes are proteins;

the normal enzyme for the metabolism of phenylalanine is a protein;

proteins are coded for by a sequence of triplet codons of bases in the DNA;

translated into mRNA;

transcribed into protein;

any change in this process e.g. mutation of DNA triplet codons;

results in lack of / non-functioning enzyme;

in this case that which metabolises phenylalanine;

excess phenylalanine accumulates;

and changes the phenotype;

(7)

Total 14

Question 10

Study the chart below setting out various heart disease risk factors then answer the questions that follow.

- a) i) Explain why the lowest total score for all the columns on the chart is not zero.
there are many unknown factors involved in heart disease;
most heart attacks not clearly correlated with risk factors;
cannot eliminate inherited risks; (2)
- ii) Calculate the percentage by which the maximum risk score can be reduced by exercise.
6/63;
 $x 100 = 9.5\%$ (2)
- iii) In the light of your answer to a) ii) above, explain whether you would advise an exercise regime for sedentary people who have been cleared by a physician to carry it out.
yes, any reduction in risk is useful;
but only of limited value; (1)
- c) Describe the types of people who would have the highest and lowest risk factors totals, and explain the advice that you would give to the one with the highest risk.
Score 63 - bald, stocky male over 61
Score 7 - female age 10 - 20,
stop smoking;
and / or reduce progressively;
gives the biggest reduction of risk of 11;
reduce the percentage of animal fats in the diet reduction of risk of up to 5;
unrealistic to hope to achieve no animal fat in diet after such a high original intake;
start a carefully monitored progressive programme of aerobic exercise;
the reduction of animal fats and progressive exercise should result in weight loss;
may be offset by increased appetite on stopping / reducing exercise;
general reassessment of calorific intake including non-fat sources;
lose no more than a kilogram per week;
keep correct balance of proteins and carbohydrates;
otherwise body protein lost with drop in body metabolism;
less calorie use;
'dieting makes you fat';
keep well hydrated;
relaxation only has marginal effects on essential hypertension (blood pressure); (7)

Total 14

Biology



1 - Molecules, Cells & Systems

A2 - Making Use of Biology

3 - Pathogens & Disease

Complete with Assessment Grids & Mark Schemes

FELTHAM PRESS

Examination Style Question Papers

Name: _____

Unit 1 - Molecules, Cells and Systems

Year Set 3

Time allowed 1 hour 30 minutes

Instructions:

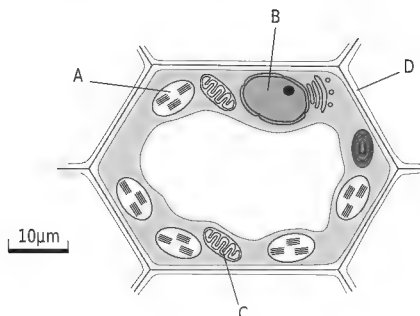
- Answer **all** the questions in the spaces provided.

Information

- Mark allocations are shown in brackets.
- The maximum mark for this paper is 75.
- just over half of the marks (43/75) will be for knowledge and understanding; just under half of the marks (32/75) will be for application of knowledge and understanding, analysis and evaluation;
- Quality of Written Communication will be assessed in answers to these questions.
- Scientific terminology should be used where appropriate.
- Calculators may be used.

Question 1

Study the diagrams below of the generalised plant cell as seen under an electron microscope and answer the questions that follow.



- a) Identify structures A to C

A _____
 B _____
 C _____

(3)

- b) Typical plant cells are described as eukaryotic cells, describe the main features of eukaryotic cells.

(2)

- c) Name a type of organism with a prokaryotic cell, and name the cell structure they have in common with eukaryotic cells.

(2)

Total 7

Question 2.

- a) In the table below list three differences between the light (optical) and electron microscopes

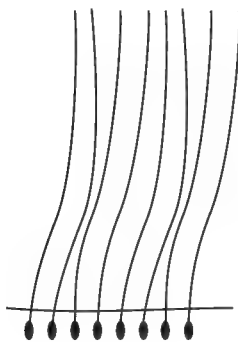
Light (optical) microscope	Electron microscope

(3)

- b) Describe the main difference between the principles of the transmission and scanning electron microscopes.

(2)

- c) Drawing X represents cilia. Drawing Y represents the appearance of a section of cilia.



X



Y

Interpret and explain the difference in appearance between the two cell diagrams.

(3)

Total 8

Question 3

Read the following passage and answer the following questions.

Osmosis can be described as the movement of water from a solution of less negative (higher) water potential to a solution of more negative (lower) water potential through a partially permeable membrane. It can also be thought of as the special case of the diffusion of water, with water molecules diffusing down a gradient from regions of its higher potential to regions of its lower potential through a partially permeable membrane. The term 'potential' is used rather than the more familiar 'concentration' when talking about diffusion, as water is the solvent. Like diffusion, the rate of the passage of water by osmosis is proportional to the steepness of the potential gradient. Pressure increases the water potential of a given aqueous system such as a living plant cell.

- a) i)** What is the water potential of pure water at standard temperature and pressure?

(1)

- ii)** Explain why alternatives to the term the 'concentration of water' have been devised.

(2)

- iii)** Name two factors (other than the one mentioned in the text) to which the rate of osmosis is proportional to.

(2)

- b)** If a plant cell is placed in pure water, it eventually becomes fully turgid, the cellulose cell wall can expand no further, and so no more water enters the cell. Explain this process in terms of the effect of pressure on water potential.

(4)

Total 9

Question 4

A solution of unknown composition is tested with Benedict's solution and a positive result is obtained.

- a) Describe a positive result with the Benedict's test.

(2)

- b) Explain why it would be wrong to deduce that the positive Benedict's test indicates that glucose is present.

(3)

- c) Explain why a solution of sucrose must undergo acid hydrolysis before a positive result for the Benedict's test can be obtained from it.

(3)

Total 8

Question 5

- a) Give the names of two proteins found in the body.

(1)

- b) Describe the Biuret test for proteins, and the appearance of a positive result.

(2)

- c) Explain how the shape of the three dimensional tertiary structure of an enzyme is essential for its role as a biological catalyst.

(3)

Total 6

Question 6

- a) Define the term organ, and name an example

(3)

- b) Blood is a specialised tissue containing a number of different cell types, give a distinguishing feature of each of the types of blood cell listed below.

- i) red blood cell

(1)

- ii) lymphocyte

(1)

- iii) monocyte

(1)

- iv) granulocyte

(1)

Total 7

Question 7

Air that is breathed in (inspired air) contains about 21% by volume of oxygen, and 0.04% by volume of carbon dioxide, and air that is breathed out (expired air) contains about 16% by volume of oxygen

- a)** Name two other main components of inspired air.

(2)

- b)** Give figures for the percentage by volume of carbon dioxide in expired air.

(1)

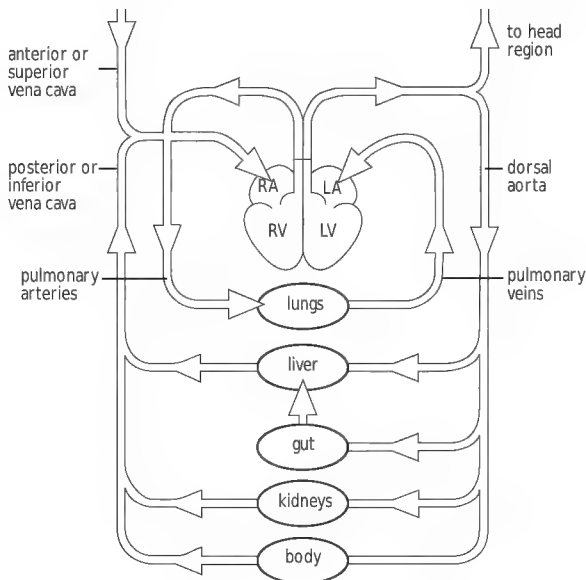
- c)** Explain the relationship between the figures for inspired air, and the figures for expired air

(2)

Total 5

Question 8

Study the diagram below of the general pattern of blood circulation in a mammal, and by reference to the diagram and your own knowledge answer the the questions that follow.



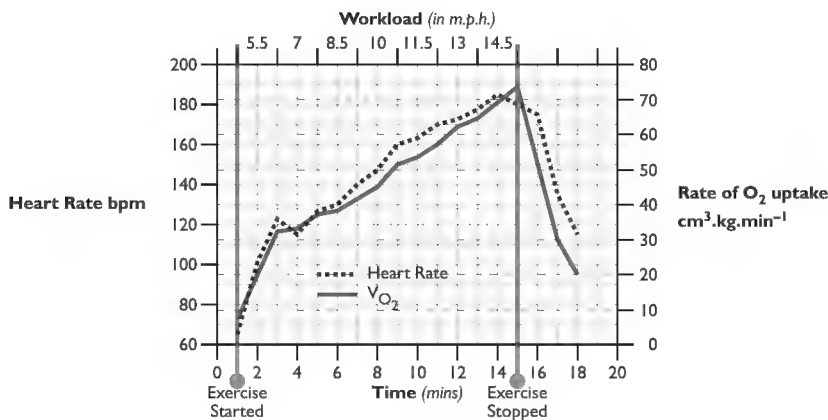
- a) Label the diagram to identify by name the blood vessels entering the liver and kidneys. (3)
- b) Describe the pathway of the blood from the right atrium of the heart to the liver, explaining the main changes that occur in the blood as a result of its passage through the various organs along its route.

(7)

Total 10

Question 9

Study the graph below showing the heart rate and oxygen uptake of a middle distance runner performing a maximal treadmill test, and answer the following questions.



a) Identify the periods over which:

i) the greatest rate of increase of heart rate and oxygen uptake occurs;

ii) the oxygen uptake continues to increase and the heart rate decreases.

(2)

b) For this individual identify the

i) resting heart rate;

ii) maximum heart rate.

(2)

Question continued...

Assessment Grid

Unit 1 - Molecules, Cells and Systems Year Set 3

Specification Section	Question Number									Total
	1	2	3	4	5	6	7	8	9	
12.1 Cell Structure	6									6
12.2 Ultrastructure	1	8								9
12.3 Membrane & Cell Transport			9							9
12.4 Biological Molecules				8	1					9
12.5 Enzymes					5					5
12.6 Tissue and Organs						7	5			12
12.7 Blood System								10		10
12.8 Heart									14	14
Total										75

Assessment Objective	Question Number									Total
	1	2	3	4	5	6	7	8	9	
AO1										
Knowledge with Understanding	6	5	5	4	3	7	3	6	3	42
AO2										
Application/Analysis/Evaluation	1	3	4	4	3		2	4	11	33
Total										75

Mark Schemes for Question Paper

Unit 1 - Molecules, Cells and Systems Year Set 3

Instructions

; = 1 mark / = alternative response

Question 1

Study the diagrams of the generalised plant and animal cells as seen under a light (optical) microscope and answer the following questions.

- a) Identify structures A to C

A = Chloroplast

B = Nucleus

C = Mitochondrion;

(3)

- b) Typical plant cells are described as eukaryotic cells, describe the main features of eukaryotic cells.

presence of a membrane bound nucleus at some stage in their existence;

membrane bound organelles, e.g. mitochondria;

(2)

- c) Name a type of organism with a prokaryotic cell, and name one cell structure that they have in common with eukaryotic cells.

bacterium;

ribosomes;

(2)

Total 7

Question 2

- a) In the table below list three differences between the light (optical) and electron microscopes

Light (optical) microscope	Electron microscope
Uses light	Uses a stream of electrons
Light focused by lenses	Electrons focused by magnets
Objects observed directly	Objects observed via fluorescent screen

(3)

- b) Explain the main difference between the principles of the transmission and scanning electron microscopes.

Transmission electron microscope - electrons pass through thin sections of specimen;

Scanning electron microscope - electrons reflected from surface of specimen;

(2)

- c) Drawing X represents cilia. Drawing Y represents the appearance of a section of cilia.

Interpret and explain the difference in appearance between the two cell diagrams.

cilia elongated relatively long thin structures;

thin sections along their length only cut small lengths;

and rarely 'catch' the connection to the cell;

giving appearance of unattached segments;

(3)

Total 8

Question 3

Read the following passage and answer the following questions.

- a) i) What is the water potential of pure water at standard temperature and pressure?
0 (nought / zero) (1)
- ii) Explain why alternatives to the term the 'concentration of water' have been devised.
water is the solvent in which solutes dissolve;
the term concentration normally refers to the solute;
therefore the 'term concentration of water' implies it is acting as a solute; (2)
- iii) Name two factors (other than the one mentioned in the text) to which the rate of osmosis is proportional.
temperature;
surface area over which it is occurring; (2)
- b) If a plant cell is placed in pure water, it eventually becomes fully turgid, the contents of a fully turgid cell push out against the stretched cell wall which can expand no further and so no more water enters the cell. Explain this process in terms of the effect of pressure on water potential.
the pressure inside the cell is raised above that in a non-turgid cell;
an increase in pressure increases the water potential;
reducing the water potential gradient to zero;
so no more water enters the cell (4)

Total 9

Question 4

A solution of unknown composition is tested with Benedict's solution and a positive result is obtained.

- a) Describe a positive result with the Benedict's test.

green / yellow/ red;

precipitate;

(2)

- b) Explain why it would be wrong to deduce that glucose was present from the positive Benedict's test.

Benedict's test is a test for reducing sugars;

there are reducing sugars other than glucose;

e.g. fructose, maltose, and lactose;

(3)

- c) Explain why a solution of sucrose must undergo acid hydrolysis before a positive result for the Benedict's test can be obtained from it.

sucrose is a non-reducing sugar;

which gives a negative result with the Benedict's test;

until after acid hydrolysis;

which releases the reducing sugars glucose and fructose to give a positive result;

(3)

Total 8

Question 5

- a) Give the names of two proteins in the human body.
any two proteins e.g. keratin, collagen, amylase; (1)
- b) Describe the Biuret test for proteins, and the appearance of a positive result.
add an equal volume of (5%) potassium hydroxide;
add 2 drops of 1% copper sulphate;
mauve or purple colour = positive; (2)
- c) Explain how the shape of the three dimensional tertiary structure of an enzyme is essential for its role as a biological catalyst.
the three dimensional tertiary structure is unique to a protein;
determines the shape of the 'active site';
specific active site needed to combine with a specific substrate; (3)

Total 6

Question 6

- a) Define the term organ, and name an example
collection / association / aggregation of different tissues;
organised for a common function;
any named example (3)
- b) Blood is a specialised tissue containing a number of different cell types, give a distinguishing feature of each of the types of blood cell listed below.
- i) red blood cell
no nucleus / no organelles / biconcave shape / full of haemoglobin (1)
- ii) lymphocyte
round non-granular cells with large round nucleus filling cell; (1)
- iii) monocyte
round / indented nucleus to one side; (1)
- iv) granulocyte
granular cytoplasm and lobed nucleus; (1)

Total 7

Question 7

Air that is breathed in (inspired air) contains about 20% by volume of oxygen, and 0.04% by volume of carbon dioxide, and air that is breathed out (expired air) contains about 16% by volume of oxygen

- a) Name two other main components of inspired air.

nitrogen;

water vapour;

(2)

- b) Give figures for the percentage by volume of carbon dioxide in expired air.

4%;

(1)

- c) Explain the relationship between the figures for inspired air, and the figures for expired air

the decrease in the oxygen content of expired air;

is balanced by an equivalent increase in carbon dioxide content;

(2)

Total 5

Question 8

Study the diagram below of the general pattern of blood circulation in a mammal, by reference to the diagram and your own knowledge answer the following the questions.

- a) Label the diagram to identify the blood vessels entering the liver and kidneys.

hepatic artery;

hepatic portal vein

renal artery

(3)

- b) Describe the pathway of the blood from the right atrium of the heart to the liver, briefly explaining the main changes that occur to the blood as a result of its passage through the various organs.

to right ventricle;

where pressure is increased;

to lungs;

where oxygen in blood increases and carbon dioxide decreases;

to left atrium;

to left ventricle where pressure increased;

to the liver;

to the gut;

where variable amounts of soluble nutrients are absorbed;

glucose, amino acids, fatty acids, glycerol, inorganic ions and water all increase;

to the liver;

(7)

Total 10

Question 9

Study the graph below showing the heart rate and oxygen uptake of a middle distance runner performing a maximal treadmill test, and answer the following questions.

- a) Identify the periods over which:
- i) the greatest rate of increase of heart rate and oxygen uptake occurs;
1 - 2 minutes / first minute of exercise (1)
 - ii) the oxygen uptake continues to increase and the heart rate decreases.
14 - 15 minutes / 13 - 14 minutes after the start of exercise (1)
- b) For this individual identify the
- i) resting heart rate;
65 - 70 bpm (1)
 - ii) maximum heart rate.
180 - 185 bpm (1)
- c) Explain why the heart rate and oxygen uptake follow each other so closely throughout this investigation.
- tissues (muscles) demand oxygen for aerobic respiration;
demand for oxygen met by increase in blood supply;
increase in blood supply achieved by increase in cardiac output which involves an increase in heart rate; (3)
- d) Interpret the overall shapes of the curves in terms of what is occurring within the athlete's body during this investigation.
- at start of exercise rapid demand for extra oxygen by muscles;
heart rate and oxygen uptake increase accordingly;
after 3 minutes of effort rates of both slow as intensity cannot be maintained;
steady increase to maximum values;
oxygen uptake continues to rise after heart rate starts to fall as a result of continuing high rate of extraction by maximally working muscles;
point of exhaustion reached and exercise stopped;
complication due to strength of motivation of athlete
slow decrease after stopping as a result of post exercise oxygen consumption (EPOC)
measurements terminated before full recovery; (7)

Total 14

Examination Style Question Papers

Name: _____

Unit 2 - Applied Biology

Year Set 3

Time allowed | hour 30 minutes

Instructions:

- Answer **all** the questions in the spaces provided.

Information

- Mark allocations are shown in brackets.
- The maximum mark for this paper is 75.
- just over half of the marks (43/75) will be for knowledge and understanding; just under half of the marks (32/75) will be for application of knowledge and understanding, analysis and evaluation;
- Quality of Written Communication will be assessed in answers to these questions.
- Scientific terminology should be used where appropriate.
- You may use a calculator.

Question 1

- a) Name two characteristics of enzymes that enable them to be used as analytical reagents.

(2)

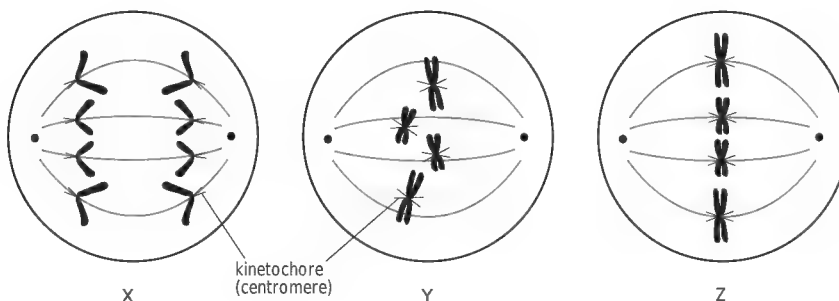
- b) The enzyme glucose oxidase catalyses the conversion of glucose to gluconic acid. Glucose oxidase immobilised in a semi-conducting silicon chip can give a measure of the level of blood glucose by the generation of a small electric current which can be converted to a liquid crystal display. Explain the way in which the conversion catalysed by glucose oxidase can result in an accurate measure of blood glucose level.

(3)

Total 5

Question 2

Study the diagrams of different stages in mitosis and answer the following questions.



- a) i) Write the letters in the correct order to show the sequence in which these stages occur in mitosis.

(1)

- ii) Describe what is occurring with respect to the chromosomes / chromatids in the stages of mitosis shown in diagrams Y and Z.

(4)

- iii) Explain the shape of the chromosomes in diagram X.

(3)

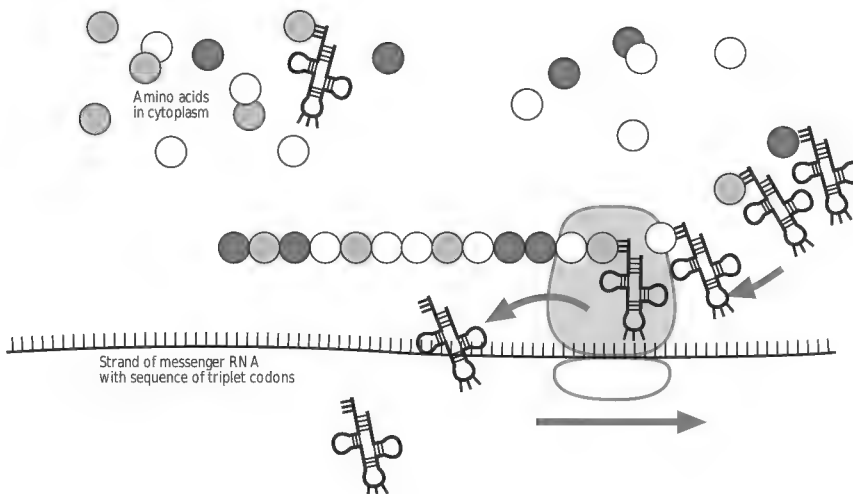
- b) Distinguish between a chromosome and a chromatid.

(4)

Total 12

Question 3

Study the diagram of protein synthesis and answer the following questions.



a) On the diagram label the following:

- i) a transfer RNA (tRNA) molecule; (1)
- ii) an anti-codon; (1)
- iii) the ribosome; (1)
- iv) the polypeptide; (1)

b) With regard to the formation of a polypeptide chain:

- i) define the term 'translation'; (1)

- ii) what is the function of the ribosome? (1)

- iii) which nucleotide base is present in mRNA but not in DNA; (1)

- iv) describe how a polypeptide chain gives rise to an active protein enzyme molecule. (3)

Total 10

Question 4

The table below summarises the effects of two antibiotics on genetically modified (engineered) bacteria. The bacteria have been exposed to plasmids either containing the two genes for resistance to Ampicillin and Tetracycline, or the gene for resistance to one antibiotic and the gene for human insulin.

Bacteria Resistant to antibiotics	Ampicillin	Tetracycline
A	No	No
B	Yes	Yes
C	Yes	No

a)

i) Describe the nature of plasmids.

(1)

ii) Describe and explain the treatment to which bacteria can be exposed to that encourages them to take up plasmids in such an experiment.

(2)

iii) From the results shown in the table above state whether bacteria A, B, and C have in terms of plasmids they have taken up.

A

B

C

(1)

b) Given the results in the table, briefly describe how bacteria carrying the gene for human insulin could be isolated from a mixed culture of A, B, and C.

(3)

Total 7

Question 5

Study the table of comparisons between weed species and non-weed species of the plant Genus *Eupatorium*

Eupatorium	Eupatorium
<i>E. microstremon</i> (weed) (n=4)	<i>E. pycnocephalum</i> (non-weed) (n=20)
Genetically variable	Genetically not very variable
Annual, quick to flower	Perennial, slow to flower
Flowers in any day length	Flowers better in short days
Self-compatible	Self-incompatible
Tolerant of drought & waterlogging	Intolerant

After T. A. Hill, The Biology of Weeds

- a) Give a definition of the term 'weed'.

(2)

- b) Explain how any **two** of the features of the weed species listed in the table contribute to its success as a weed.

(4)

Total 6

Question 6

The table below shows the bioaccumulation of a pesticide in a marine food chain.

Component of food chain	Concentration of pesticide in ppm (parts per million)
Sea water	0.00005
Zooplankton	0.04000
Shrimp	0.16000
Fish	1.33000
Sea Bird	75.5000

- a) Describe what is meant by the term 'bioaccumulation'.

(1)

- b) Explain how bioaccumulation of persistent (long lasting) pollutants occurs in a food chain.

(3)

- c) i) Identify the prey and the predator between which the greatest increase in concentration of pesticide occurs.

(1)

- ii) Calculate the multiplication factor that has occurred between the two animals that you have identified.

(1)

- iii) Explain why the greatest increase in concentration occurs between these two animals.

(2)

Total 8

Question 7

Under conditions of high light intensity the natural levels of carbon dioxide (0.04%) in the atmosphere limit the rate of photosynthesis, and thus the productivity of crop plants.

- a) Explain why increasing the level of carbon dioxide in glasshouses increases the productivity of glasshouse crop plants such as tomatoes.

(3)

- b) Some figures for the increase in productivity of tomatoes as a result of increasing carbon dioxide concentration are given below.

% carbon dioxide concentration in the atmosphere	% increase in tomato crop productivity
0.06	+ 20
0.09	+ 36
0.12	+ 39

Using these figures explain what advice you could give to growers with respect to the most cost efficient level to which carbon dioxide can be increased in their greenhouses.

(2)

- c) Explain why growers of greenhouse tomato crops are advised to stop increasing the carbon dioxide levels in their greenhouses during hot summers.

(3)

Total 8

Question 8

The technique of genetic fingerprinting may be used to identify individual blood samples.

- a) i) Identify from which part of a blood sample the DNA is extracted, and explain why.

(1)

- ii) Explain which part of the DNA is used to generate the “DNA fingerprints”.

(2)



- iii) A DNA fingerprint is shown below.

Identify the best match from A, B, C, and D.

(1)

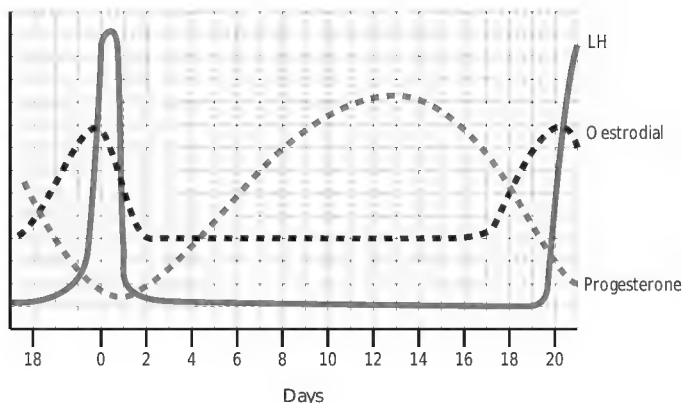
- b) Explain why, although the chances of any two individuals (apart from identical twins) having matching DNA fingerprints are virtually impossible, there are still reservations about the use of the technique in forensic science.

(6)

Total 10

Question 9

The oestrous cycle of dairy cows is shown below.



- a) i) From the diagram above determine the length of the oestrus cycle.

(1)

- ii) Give a brief description of the variations in the levels of luteinising hormone (LH) during this cycle.

(2)

- iii) On the diagram indicate the time of ovulation in this cycle.

(1)

- b) Explain why the detection of oestrus, or the period of 'heat', in dairy cows is of commercial significance.

(6)

Total 10

Assessment Grid

Unit 2 - Applied Biology Year Set 3

Specification Section	Question Number									Total
	1	2	3	4	5	6	7	8	9	
11.1 Enzymes	6									6
11.2 Mitosis		10								10
11.3 DNA			10							10
11.4 Gene technology				7						7
11.5 Forensic science								10		10
11.6 Plant growth					6	8	8			22
11.7 Biotechnology/Reproduction									10	10
Total										75

Assessment Objective	Question Number									Total
	1	2	3	4	5	6	7	8	9	
AO1										
Knowledge with Understanding	3	6	10	4	2	7	5	3	3	43
AO2										
Application/Analysis/Evaluation	3	4		3	4	1	3	7	7	32
Total										75

Mark Schemes for Question Paper

Unit 2 - Applied Biology **Year Set 3**

Instructions

; = 1 mark / = alternative response

Question 1

- a) Name two characteristics of enzymes that enable them to be used as analytical reagents.

high sensitivity;

specificity;

(2)

- b) The enzyme glucose oxidase catalyses the conversion of glucose to gluconic acid. Glucose oxidase immobilised in a semi-conducting silicon chip can give a measure of the level of blood glucose by the generation of a small electric current which can be converted to a liquid crystal display. Explain the way in which the conversion catalysed by glucose oxidase can result in an accurate measure of blood glucose level.

gluconic acid dissociates to release hydrogen ions;

hydrogen ions result in an electrical current;

proportional to the amount of acid;

in turn proportional to the amount of glucose present / converted;

(3)

Total 5

Question 2

Study the diagrams of different stages in mitosis and answer the following questions.

- a) i) Write the letters in the correct order to show the sequence in which these stages occur in mitosis.

Y, Z, X

(1)

- ii) Describe what is occurring with respect to the chromosomes / chromatids in the stages of mitosis shown in diagrams Y and Z.

Y - the chromosomes (each of two chromatids) are just formed;

and moving towards the equator of the nuclear spindle apparatus;

Z - the chromosomes (each of two chromatids) are arranged on the equator;

by means of the kinetochore (centromere) attached to fibres of the spindle apparatus;

(4)

- iii) Explain the shape of the chromosomes in diagram X.

the kinetochores (centromeres) are the centres of movement of the chromosomes;

they lead the way along the spindle fibres towards the centrioles at the poles of the spindle;

the 'arms' of the chromosomes trail behind;

(3)

- b) Distinguish between a chromosome and a chromatid.

a chromosome contains a haploid (single 'dose') of DNA;

chromosomes appear (condense) containing a diploid (double 'dose') of DNA;

organised into two duplicate parts - chromatids;

sharing a common kinetochore;

chromatids become new 'daughter' chromosomes when the kinetochore divides;

(4)

Total 12

Question 3

Study the diagram below of protein synthesis and answer the following questions.

- a) On the diagram label the following:
- i) a transfer RNA (tRNA) molecule; (1)
 - ii) an anti-codon; (1)
 - iii) the ribosome; (1)
 - iv) the polypeptide; (1)
- b) With regard to the formation of a polypeptide chain:
- i) define the term 'translation';
the conversion of the genetic code on the mRNA into the reality of a polypeptide chain; (1)
 - ii) what is the function of the ribosome?
to organise synthesis of the polypeptide chain; (1)
 - iii) which nucleotide base is present in mRNA but not DNA?
uracil; (1)
 - iv) describe how a polypeptide chain gives rise to an active protein enzyme molecule.
as it gets longer, attractions between parts of the chain;
e.g. forming hydrogen bonds;
result in folding of chain;
complex three dimensional shape formed with an active site; (3)

Total 10

Question 4

The table below summarises the effects of two antibiotics on genetically modified (engineered) bacteria. The bacteria have been exposed to plasmids either containing the two genes for resistance to Ampicillin and Tetracycline, or the gene for resistance to one antibiotic and the gene for human insulin.

Bacteria Resistant to antibiotics	Ampicillin	Tetracycline
A	No	No
B	Yes	Yes
C	Yes	No

- a) i) Describe the nature of plasmids.
circular / ring like DNA in bacterial cytoplasm; (1)
- ii) Describe and explain the treatment to which bacteria can be exposed to that encourages the uptake of plasmids in such an experiment.
ice-cold calcium chloride;
after 15 minutes in ice 90 seconds at 42°C; (2)
- iii) From the results shown in the table above state whether bacteria A, B, and C in terms of plasmids they have taken up.
A none taken up
B unaltered plasmids taken up
C plasmids with gene for human insulin taken up; (1)
- b) Given the results in the table, briefly describe how bacteria carrying the gene for human insulin could be isolated from a mixed culture of A, B and C.
plate out on agar plates containing ampicillin;
B and C will grow;
replica plate onto agar plates containing both antibiotics;
only B colonies will grow on this;
return to previous plate and take sample from colony(ies) of C; (3)

Total 7

Question 5

Study the table of comparisons between weed species and non-weed species of the plant Genus *Eupatorium*.

- a) Give a definition of the term 'weed'.
a plant that succeeds in cultivated soil;
and competes with cultivated plants; (2)
- b) Explain how any two of the features of the weed species listed in the table contribute to its success as a weed.
- Genetically variable
variation in offspring increases chance of survival;
of the 'fittest' in the 'struggle for existence';
when environment undergoes rapid change as it does during cultivation;
- Annual, quick to flower
completes life cycle / produces seed;
between harvests of crop and ploughing;
- Flowers in any day length
can flower independent of season;
when conditions have promoted rapid growth early in the year;
or later after a second germination in the season;
- Self-compatible
can self-fertilise;
no need for cross pollination agents;
- Tolerant of drought and waterlogging
survive extreme conditions;
better than crops; (4)

Total 6

Question 6

The table below shows the bioaccumulation of a pesticide in a marine food chain.

Component of food chain	Concentration of pesticide in ppm (parts per million)
Sea water	0.00005
Zooplankton	0.04000
Shrimp	0.16000
Fish	1.33000
Sea Bird	75.5000

- a) Describe what is meant by the term 'bioaccumulation'.
the concentration of a substance in a living organism;
above that of the food or water that it takes in; (1)
- b) Explain how bioaccumulation of persistent(long lasting) toxins (poisons) occurs in a food chain.
organisms have to take in large amounts of substances in their nutrition;
if toxins are present they can be absorbed by the tissues;
especially if they are fat soluble;
feeding organism acts like a 'biological filter'; (3)
- c) i) Identify the prey and predator between which the greatest increase in concentration of pesticide occurs.
sea bird and fish (1)
- ii) Calculate the multiplication factor that has occurred between the two animals that you have identified.
56.77 times (1)
- iii) Explain why the greatest increase in concentration occurs between these two animals.
sea bird takes in a greater relative mass of food;
than other predators in the chain;
bird has fat reserves in its body; (2)

Total 8

Question 7

Under conditions of high light intensity the natural levels of carbon dioxide (0.04%) in the atmosphere limit the rate of photosynthesis, and thus the productivity of crop plants.

- a) Explain why increasing the level of carbon dioxide in glasshouses increases the productivity of glasshouse crop plants such as tomatoes.
- increases photosynthesis;
increases excess of photosynthesis over respiration (productivity);
therefore more organic matter available for crop production;

(3)

- b) Some figures for the increase in productivity of tomatoes as a result of increasing carbon dioxide concentration are given below.

% carbon dioxide concentration in the atmosphere	% increase in tomato crop productivity
0.06	+ 20
0.09	+ 36
0.12	+ 39

Using these figures explain what advice you could give to growers with respect to the most cost efficient level to which carbon dioxide can be increased in their greenhouses.

the first 0.03% increase to 0.09 increases yield by +16%;
the second from 0.09 to 0.12 increases yield only by +3%;
law of diminishing returns;

(2)

- c) Explain why growers of greenhouse tomato crops are advised to stop increasing the carbon dioxide levels in their greenhouses during hot summers.
- when hot ventilators must be opened to avoid overheating;
carbon dioxide would be lost;
uneconomic balance;
also any water stress would lead to stomatal closure;

(3)

Total 8

Question 8

The technique of genetic fingerprinting may be used to identify individual blood samples.

- a) i) Identify from which part of a blood sample the DNA is extracted, and explain why.
white cells as they are the only component of blood containing DNA; (1)
- ii) Explain which part of the DNA is used to generate the fingerprints.
non-coding part / introns;
minisatellites / variable number tandem repeats / VNTR; (2)
- iii) A DNA fingerprint is shown below.
Identify the best match from A, B, C, and D.
C (1)
- b) Explain why, although the chances of any two individuals (apart from identical twins) having matching DNA fingerprints are virtually impossible, there are still reservations about the use of the technique in forensic science.
difficult to obtain pure samples of DNA;
contamination from bacteria / fungi / microorganisms;
could generate false matches;
DNA can degrade into fragments of variable length which confuse technique;
charged ions from contaminants can affect separation of charged fragments of DNA;
human error in sampling;
some forensic laboratories have not followed correct procedures;
criminals may contaminate scene of crime with false samples; (6)

Total 10

Question 9

The oestrous cycle of dairy cows is shown below.

- a) i) From the diagram above determine the length of the oestrus cycle.
21 days; (1)
- ii) Give a brief description of the variations in the levels of luteinising hormone (LH) during this cycle.
peaks at 0 and 21 days;
low constant level between these peaks; (2)
- iii) On the diagram indicate the time of ovulation in this cycle.
12-15 hours after oestrus / at peak of LH; (1)
- b) Explain why the detection of oestrus, or the period of 'heat', in dairy cows is of commercial significance in UK Dairy farming.
more than 70% of fertilisations are by artificial insemination (AI);
must identify correct period of heat;
and subsequent ovulation;
to maximise success of AI;
which ensures good progeny / offspring;
if inaccurate success rate of AI reduced to 2-10%; (6)

Total 10

Examination Style Question Papers

Name: _____

Unit 3- Pathogens and Disease

Year Set 3

Time allowed | hour 30 minutes

Instructions:

- Answer **all** the questions in the spaces provided.

Information

- Mark allocations are shown in brackets.
- The maximum mark for this paper is 75.
- just over half of the marks (43/75) will be for knowledge and understanding; just under half of the marks (32/75) will be for application of knowledge and understanding, analysis and evaluation;
- Quality of Written Communication will be assessed in answers to these questions.
- Scientific terminology should be used where appropriate.
- You may use a calculator.

Question 1

The association of disease with microorganisms was only established in the 19th Century by pioneer researchers such as Pasteur and Koch. Koch who demonstrated that TB was caused by 'germs' (*Mycobacterium tuberculosis*) established three 'postulates' that must be fulfilled to establish the link between microorganisms and disease.

a) List the three Koch's postulates.

i) _____ (1)

ii) _____ (1)

iii) _____ (1)

b) Explain the problems caused by viruses to the early workers trying to establish the 'germ' theory of infectious disease.

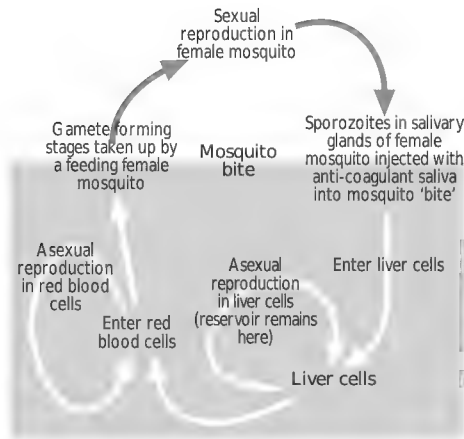
_____ (3)

Total 6

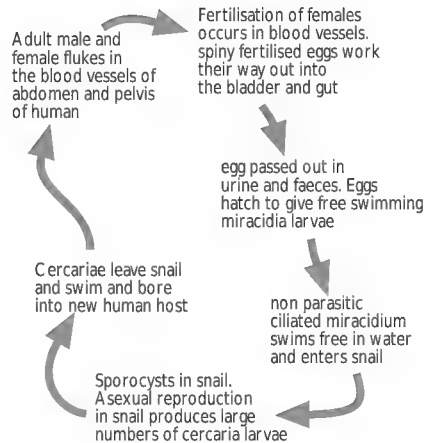
Question 2

Plasmodium and *Schistosoma* are both blood parasites of humans, and simplified life cycles are set out below.

Plasmodium



Schistosoma



- a) In parasites with two hosts in their life cycle, the host in which sexual reproduction occurs is known as the primary host, and the other as the secondary host. Identify the primary hosts in the two life cycles and comment on your observations.

(2)

- b) i) Describe the way in which the two parasites differ in their mode of transmission between human hosts.

(2)

- ii) Explain how one of these modes of transmission could be considered to be more efficient than the other.

(2)

Total 6

Question 3

a) With regard to the acquisition of immunity, explain the following terms.

i) Natural active immunity.

(1)

ii) Artificial active immunity.

(2)

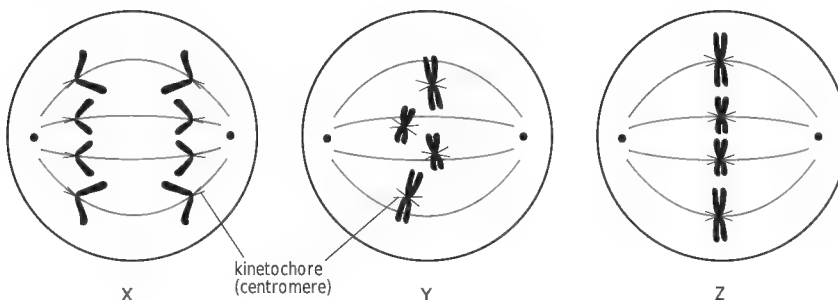
b) Suggest reasons why it has proved difficult to develop a vaccine against organisms such as the malarial parasite

(2)

Total 5

Question 4

Study the diagrams of different stages in mitosis shown below then answer the questions that follow.



- a) i) Write the letters in the sequence that these stages occur in mitosis.

(1)

- b) Describe what is occurring with respect to the chromosomes / chromatids in stages Y and Z shown in the diagram.

i) Y

(1)

ii) Z

(1)

iii) Explain the shape of the chromosomes in diagram X.

(2)

- c) Distinguish between a chromosome and a chromatid.

(2)

Total 7

Question 5

Cystic fibrosis is a serious condition caused by the lack of a correctly functioning transmembrane protein which controls the transport of chloride ions across the cell membrane.

- a) Explain how the lack of a correctly functioning transmembrane protein can arise from a deletion of three nucleotides in the DNA.

(3)

- b) Explain how the lack of a correctly functioning transmembrane protein which controls the transport of chloride ions across the cell membrane influences the phenotype.

(2)

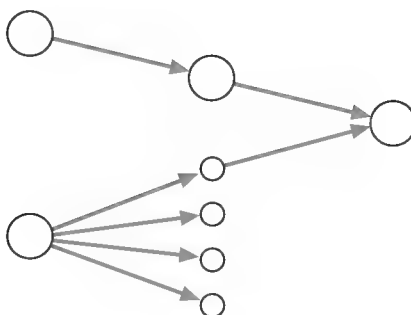
Total 5

Question 6

- a) Explain, in terms of chromosome numbers, the importance of meiosis in sexual reproduction.

(3)

- b) In some organisms it is not unusual for unreduced gametes to be produced, that is gametes with the diploid number of chromosomes. Using the circles below representing the nuclei of gametes and fertilised eggs, demonstrate what the chromosome number of the offspring formed from an unreduced egg and a normal spermatozoa would be.



(2)

Total 5

Question 7

- a) DNA 'probes' are used to detect specific lengths of other strands of DNA by hybridisation. The probe is complementary to the one being detected. The diagram below represents short lengths of DNA and a DNA probe.

- i) Match the probe with the correct strand of DNA.

DNA strands;

AATCGGCCTTATGCCGGT

AATAGGCTTTATGCAGGT

AATAGGCTGTATGCCGGT

DNA probe;

ATACGTC

(1)

- ii) Give two ways in which the position of a successful hybridisation can be detected.

(2)

- b) Explain the role of genetically engineered bacteria in the technique.

(3)

Total 6

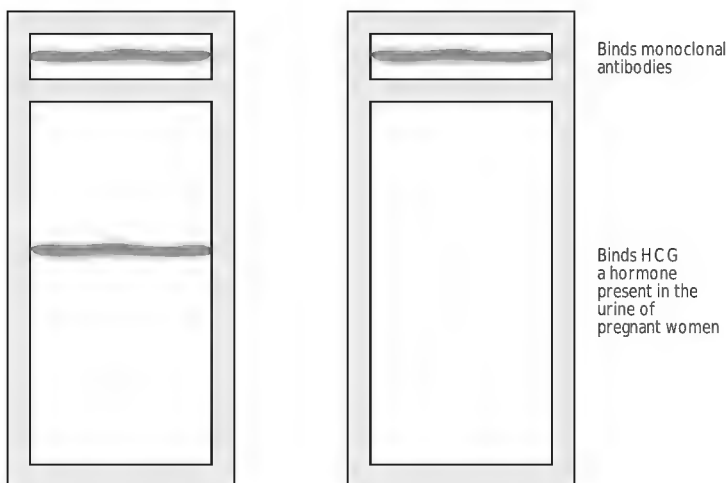
Question 8

Each type of B-lymphocyte of the human immune system secretes only one type of antibody. There are many uses for pure preparations of single antibodies (monoclonal antibodies), but B-lymphocytes are difficult to culture.

- a) Explain how this problem has been overcome by cell hybridisation.

(3)

- b) One diagnostic use of monoclonal antibodies is in the pregnancy testing strip. Study the diagrams of two pregnancy testing strips then answer the questions that follow.



Explain the need for the upper small window on the test strips.

(2)

Total 5

Question 9

The production of human insulin (and other proteins) from single celled organisms such as bacteria. requires the isolation of the relevant human gene as the first stage.

- a)** Describe which human cells it is best to use as a source of the human gene for insulin, and explain why.

(2)

- b) i)** Explain why it is better to isolate the mRNA from these cells, and not to try and isolate the actual length of DNA that codes for the insulin.

(2)

- ii)** Describe the action of the enzyme reverse transcriptase in this process of gene isolation.

(1)

- c)** Before the success of genetically engineering the human gene for insulin, insulin was obtained by extracting it from the pancreases of animals such as the pig. Describe the advantages of producing human hormones from engineered bacteria, rather than extracting them from animal organs.

(3)

- d)** Summarise the moral and ethical issues associated with the use of recombinant DNA techniques.

(7)

Total 15

Question 10

Much interest centres around the role and importance of genetic factors in the incidence of disease. Some diseases are inherited as a result of changes in the genetic material, but it is also clear that different people have different susceptibilities to diseases that are caused by environmental factors such as infectious agents, carcinogens and radiation.

- a)** A recent investigation into the incidence of the skin cancer melanoma in the UK, of which there are about 4500 cases with 1500 fatalities per year, involved a study of 450 pairs of twins. Explain why twin studies are important in research trying to establish the degree of genetic involvement in such a condition.

(3)

- b)** There are two main types of twins, identical twins which develop from the division of a single fertilised egg, and non-identical twins which develop from the simultaneous fertilisation of two separate eggs. This research involved 127 pairs of identical twins and 223 pairs of non-identical twins. Explain the significance of this fact to the study.

(3)

- c) i)** It is known that the more skin moles a person has the higher their risk of melanoma. The study established that identical twins were more likely to have the same number of moles and freckles than non-identical twins. Explain how this relates to assessing the genetic contribution to melanoma.

(3)

- ii)** It is suggested that genetic factors may contribute as much as 85% to the risk of contracting melanoma, given exposure to sunlight (ultra-violet radiation) of a certain intensity. Using this knowledge explain the advice you would give to people about the precautions they should take to avoid developing this condition.

(6)

Total 15

Assessment Grid

Unit 3 - Pathogens and Disease Year Set 3

	Question Number										
Specification Section	1	2	3	4	5	6	7	8	9	10	Total
12.1 Microorganisms	6										6
12.2 Parasites		6									6
12.3 Immunology			5								5
12.4 Mitosis				7		5					12
12.5 DNA					5						5
12.6 Gene technology									15		15
12.7 CHD and cancer										15	15
12.8 Diagnosis							6				6
12.9 Drugs								5			5
											Total 75

	Question Number										
Assessment Objective	1	2	3	4	5	6	7	8	9	10	Total
AO1 Knowledge with Understanding	3	4	3	5	3	3	5	3	8	8	45
AO2 Application/Analysis/ Evaluation	3	2	2	2	2	2	1	2	7	7	30
											Total 75

Mark Schemes for Question Paper

Unit 3- Pathogens and Disease Year Set 3

Instructions

; = 1 mark / = alternative response

Question 1

The association of disease with microorganisms was only established in the 19th Century, by pioneer researchers such as Pasteur and Koch. Koch who demonstrated that TB was caused by 'germs' (*Mycobacterium tuberculosis*) established three 'postulates' that must be fulfilled to establish the link between microorganisms and disease.

- a) List the three Koch's postulates.
- i) microbes must invariably be present in the diseased organism; (1)
 - ii) microbes must be capable of being cultivated outside of the host; (1)
 - iii) microbe must produce disease when injected into healthy animal; (1)
- b) Explain the problems caused by viruses to the early workers trying to establish the 'germ' theory of infectious disease.
- not visible under the light microscope;
 - difficult to purify;
 - difficult to culture outside the host;
 - impossible to culture outside of living cells; (3)

Total 6

Question 2

Plasmodium and *Schistosoma* are both blood parasites of humans, and simplified life cycles are set out below.

- a) In parasites with two hosts in their life cycle, the host in which sexual reproduction occurs is known as the primary host, and the other as the secondary host. Identify the primary hosts in the two life cycles and comment on your observations.

Plasmodium primary host mosquito;

Schistosoma primary host human;

invertebrate and vertebrate reversed;

humans could be considered to be reservoir for infection of mosquitoes;

(2)

- b) i) Describe the way in which the two parasites differ in their mode of transmission between human hosts.

Plasmodium actively injected into, and sucked out of, human hosts by the vector mosquito;

Schistosoma eggs released to external environment;

free swimming larval stages seek out hosts;

(2)

- ii) Explain how one of these modes of transmission could be considered to be more efficient than the other.

Plasmodium, no wastage,

parasite never exposed to hostile external environment;

vector actively seeks secondary host;

(2)

Total 6

Question 3

- a) With regard to the acquisition of immunity, explain the following terms.
- i) Natural active immunity.
antibodies produced as a result of natural exposure to the disease causing agent; (1)
- ii) Artificial active immunity.
antibodies produced as a result artificial exposure to a harmless dose of the disease agent;
diluted / attenuated live pathogen / dead pathogen / toxin of pathogen; (2)
- b) Suggest reasons why it has proved difficult to develop a vaccine against certain organisms such as the malarial parasite
- high mutation rate;
parasite mainly intracellular ie protected;
non-symptomatic carriers;
pathogen easily and widely spread by vector; (2)

Total 5

Question 4

Study the diagrams of different stages in mitosis then answer the questions that follow.

- a) Write the letters in the sequence that these stages occur in mitosis.
Y, Z, X (1)
- b) Describe what is occurring with respect to the chromosomes / chromatids in each of the stages shown in diagrams Y and Z.
- i) **Y** - the chromosomes (each of two chromatids) are just formed;
and moving towards the equator of the nuclear spindle apparatus; (1)
- ii) **Z** - the chromosomes (each of two chromatids) are arranged on the equator;
by means of the kinetochore (centromere) attached to fibres of the spindle apparatus; (1)
- iii) Explain the shape of the chromosomes in diagram X.
the kinetochores (centromeres) are the centres of movement of the chromosomes;
they lead the way along the spindle fibres towards the centrioles at the poles of the spindle;
the 'arms' of the chromosomes trail behind; (2)
- c) Distinguish between a chromosome and chromatids.
a chromosome contains a haploid (single 'dose') of DNA;
chromosomes appear (condense) containing a diploid (double 'dose') of DNA;
organised into two duplicate parts - chromatids;
sharing a common kinetochore;
chromatids become new 'daughter' chromosomes when the kinetochore divides; (2)

Total 7

Question 5

Cystic fibrosis is a serious condition caused as a result of the lack of a correctly functioning transmembrane protein which controls the transport of chloride ions across the cell membrane.

- a) Explain how the lack of a correctly functioning transmembrane protein can arise from a deletion of three nucleotides in the DNA.

triplet codon codes for an amino acid;

deletion of codon results in an amino acid missing from the sequence in a polypeptide / protein;

missing amino acid results in change of structure of protein;

in this case the transmembrane protein which controls the transport of chloride ions; (3)

- b) Explain how the lack of a correctly functioning transmembrane protein which controls the transport of chloride ions across the cell membrane can influence the phenotype.

transport of chloride ions followed by passive movement of water by osmosis;

lack of transport of chloride ions results in 'dry' membranes / thick mucus;

the phenotype of cystic fibrosis; (2)

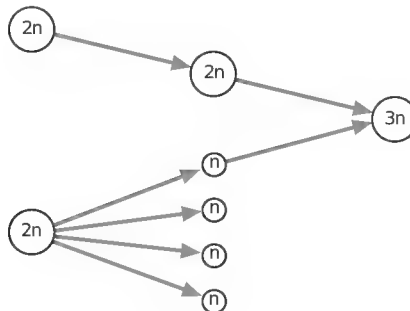
Total 5

Question 6

- a) Explain, in terms of chromosome numbers, the importance of meiosis in sexual reproduction.
- meiosis halves the chromosome number;
 - in the gametes compared to body cells;
 - typically from diploid to haploid;
 - fertilisation of haploid gametes restores diploid number;
 - in the next generation;

(3)

- b) In some organisms it is not unusual for unreduced gametes to be produced, that is gametes with the diploid number of chromosomes. Using the circles below representing the nuclei of gametes and fertilised eggs demonstrate what the chromosome number of the offspring formed from an unreduced egg and a normal spermatozoa would be like.



(2)

Total 5

Question 7

- a) DNA 'probes' are used to detect specific lengths of other strands of DNA by hybridisation. The probe is complementary to the one being detected. The diagram below represents short lengths of DNA and a DNA probe.

- i) Match the probe with the correct strand of DNA.

DNA strands;

AATCGGCCTTATGCCGGT AATAGGCTTTATGCAGGT AATAGGCTGTATGCGGGT

DNA probe; **ATACGTC** (1)

- ii) Give two ways in which the position of a successful hybridisation can be detected.

label probe with fluorescent dye;

radioactive atom; (2)

- b) Explain the role of genetically engineered bacteria in the technique.

DNA probe initially in small supply;

DNA probe introduced into genetically engineered bacteria;

which multiply copies of the DNA probe in their asexual reproduction; (3)

Total 6

Question 8

Each type of B-lymphocyte of the human immune system secretes only one type of antibody. There are many uses for pure preparations of single antibodies (monoclonal antibodies), but B-lymphocytes are difficult to culture.

- a) Explain how this problem has been overcome by cell hybridisation.
lymphocyte fused with mouse tumour cell;
hybridoma;
actively divide and produce a clone of continuously dividing antibody producing cells; (3)
- b) One diagnostic use of monoclonal antibodies is in the pregnancy testing strip. Study the diagrams of two pregnancy testing strips then answer the questions that follow.
Explain the need for the upper small window on the test strips.
without proof that the antibodies had reached the upper window;
the absence of a line in the first window could be due to non-movement or absence of antibodies; (2)

Total 5

Question 9

The production of human insulin (and other proteins) from single cell organisms such as bacteria requires the isolation of the relevant human gene as the first step.

- a) Describe which human cells it is best to use as a source of the human gene for insulin, and explain why.
beta cells of Islets of Langerhans;
as produce and secrete insulin into the blood stream; (2)
- b) i) Explain why it is better to isolate the mRNA from these cells, and not to try and isolate the actual length of DNA that codes for the insulin.
DNA of these cells contains all genes of organism;
much mRNA in these cells is for the production of insulin; (2)
- ii) Describe the action of the enzyme reverse transcriptase in this process of gene isolation.
copies DNA from mRNA; (1)
- c) Before the success of genetically engineering the human gene for insulin, insulin was obtained by extracting it from the pancreases of animals such as the pig. Describe the advantages of producing human hormones from engineered bacteria, rather than extracting them from animal organs.
engineered products pure;
no problems of contaminants eg prions / viruses etc;
known strengths;
production independent of other markets; (3)
- d) Summarise the moral and ethical issues associated with the use of recombinant DNA techniques.
general objection of 'playing God' / tampering with 'nature';
possibility of contamination with rogue genes;
possibility of escape of engineered genes into environment;
with unforeseen circumstances;
very difficult to control the technology;
small scale relatively cheap;
could be used by deviant group;
'germ' warfare possibilities;
any other valid points; (7)

Total 15

Question 10

Much interest centres around the role and importance of genetic factors in the incidence of disease. Some diseases are inherited as a result of changes in the genetic material, but it is also clear that different people have different susceptibilities to various diseases that are caused by environmental factors such as infectious agents, carcinogens and radiation.

- a) A recent investigation into the incidence of the skin cancer melanoma in the UK, of which there are about 4500 cases with 1500 fatalities per year, involved a study of 450 pairs of twins. Explain why twin studies are important in research trying to establish the degree of genetic involvement in a such a condition.

in trying to assess the degree of genetic involvement in a condition it is necessary to 'standardise' the genetic component;

twins share common genetic make-up;

(3)

- b) There are two main types of twins, identical twins which develop from the division of a single fertilised egg, and non-identical twins which develop from the simultaneous fertilisation of two separate eggs (this study involved 127 pairs of identical twins and 223 pairs of non-identical twins). Explain the significance of this fact to the study.

identical twins have identical genetic make up;

and (if not separated) share similar environmental influences

non-identical twins only share 50% of genotype;

but (if not separated) share similar environmental influences;

allows an estimation of the contribution of genetic factors;

(3)

- c) i) It is known that the more skin moles a person has the higher their risk of melanoma. The study established that identical twins were more likely to have the same number of moles and freckles than non-identical twins. Explain how this relates to assessing the genetic contribution to melanoma.

number of moles and freckles genetically determined;

melanoma related to number of moles and freckles;

the two are correlated;

(3)

- ii) It is suggested that genetic factors may contribute as much as 85% to the risk of contracting melanoma, given exposure to sunlight (ultra-violet radiation) of a certain intensity. Using this knowledge explain the advice you would give to people about the precautions they should take to avoid developing this condition.

advise susceptible group to take care to avoid over-exposure to UV light;

e.g. red haired and fair skinned people who do not tan easily;

and those with moles and freckles;

these precautions would reduce the environmental risk factors;

also advise low risk group similarly;

% risk factors apply to populations not individuals;

can still contract condition if in low risk group;

(6)

Total 15